Journal OF THE Fisheries Research Board OF Canada

Volume 31, No. 8, August 1974 **Volume 31, Nº 8, août 1974**

Environment Canada
Fisheries and Marine Service

Environnement Canada
Service des pêches et des sciences de la mer

Journal
of the
Fisheries
Research
Board
of
Canada

Environment Canada
Fisheries and Marine Service
Office of the Editor

Environnement Canada
Service des pêches et des sciences de la mer
Bureau du Rédacteur

116 Lisgar, Ottawa K1A 0H3

Volume 31, No. 8, August 1974

This number was issued on August 23, 1974.

The *Journal of the Fisheries Research Board of Canada* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, and *Journal of the Biological Board of Canada*, 1934–37.

Editorial policy — The *Journal* publishes original research articles and notes, critical reviews, essays of opinion or hypothesis, letters, and book reviews in the broad field of fisheries management, ocean science, and aquatic environments relevant to Canada. Submissions in English or French are accepted without limitations as to origin, but must not have been published or copyrighted and must not be published elsewhere except in abstract form or by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. Details on submissions are given in guides to authors and typists at the back of this issue. Reprints of these guides are available free from the Office of the Editor.

A charge of $30.00 per page is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Subscriptions — The *Journal* is issued monthly to form annual volumes of twelve numbers. Subscription rates are: Canada, $15.00 per year; other countries, $18.00. Single issues are $1.50 and $1.75, respectively. Orders must be sent directly to Information Canada, Ottawa, Canada K1A 0S9, and payment made in Canadian funds to "The Receiver General of Canada."

Microfilm — Issues from 1934 through the current volume can be purchased on 16 or 35 mm microfilm. Photocopies of individual articles or issues can be purchased. Address orders to Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Volume 31, N° 8, août 1974

Ce numéro fut publié le 23 août 1974

Publié depuis 1901, le *Journal of the Fisheries Research Board of Canada* a paru sous plusieurs titres: *Contributions to Canada Biology*, 1901–25, *Contributions to Canadian Biology and Fisheries*, 1926–34, et *Journal of the Biological Board of Canada*, 1934–37.

Politique de publication — Le *Journal* publie des articles de recherches, ainsi que des notes, critiques, exposés d'opinions ou d'hypothèses, lettres et recensions concernant le vaste domaine de la gestion des pêches, des sciences de la mer et des milieux aquatiques au Canada. Toutes contributions en français ou en anglais sont acceptées sans restriction quant à la provenance, mais elles ne doivent pas avoir été produites ni avoir fait l'objet d'un copyright et ne doivent pas être publiées nulle part ailleurs sauf sous forme abrégée ou avec le consentement écrit du directeur. Le *Journal* décline toute responsabilité pour ce qui est des affirmations des collaborateurs. Les modalités de présentation des textes font l'objet du guide des auteurs et du guide des dactylos paraissant à la fin du présent numéro. On peut se procurer gratuitement, du bureau du directeur, des exemplaires de ces guides.

Les frais de publication sont de $30.00 la page. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés.

Abonnement — Le Journal paraît tous les mois, formant ainsi un volume de 12 numéros par année. Les frais d'abonnement annuel sont de $15 pour le Canada et de $18 pour les autres pays. Chaque numéro coûte $1.50 au Canada et $1.75 à l'étranger. Prière d'adresser directement les commandes à Information Canada, Ottawa (Ontario), Canada K1A 0S9; les paiements doivent être faits en devises canadiennes, à l'ordre du Receveur général du Canada.

Microfilms — On peut acheter sur microfilms 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente. Prière d'adresser les commandes à Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, E.-U.

Editor and/Rédacteur et
Director of Scientific Information
Directeur de l'information scientifique — J. C. STEVENSON, PH.D.

Deputy Editor
Sous-rédacteur — J. WATSON, PH.D.

Assistant Editors
Rédacteurs adjoints — R. H. WIGMORE, M.SC.
JOHANNA M. REINHART, M.SC.

Production/Documentation — J. CAMP
G. J. NEVILLE/PAULA IRVING

French Abstracts/Résumés français — Y. JEAN, PH.D.

Volume 31, No. 8, August 1974
Volume 31, N° 8, Août 1974

Journal
of the Fisheries
Research
Board
of Canada

Physiological and Ecological Significance of the Chemical Instability of Uric Acid and Related Purines in Sea Water and Marine Algal Culture Medium[1]

Naval J. Antia and Arthur F. Landymore[2]

Department of the Environment, Fisheries and Marine Service
Vancouver Laboratory, Vancouver, B.C. V6T 1X2
and
Department of Zoology
University of British Columbia, Vancouver, B.C.

Antia, N. J., and A. F. Landymore. 1974. Physiological and ecological significance of the chemical instability of uric acid and related purines in sea water and marine algal culture medium. J. Fish. Res. Board Can. 31: 1327–1335.

The chemical instability of uric acid and related purine bases in a seawater phytoplankton culture medium was evaluated spectrophotometrically from concentration changes incurred on aseptic incubation at about 20 C under cool-white light or complete darkness. Uric acid showed slow degradation in darkness, which was increased severalfold by illumination. Xanthine showed a small degree of degradation only from illumination, but adenine, guanine, and hypoxanthine appeared to be stable under both test conditions. The degradation of uric acid was traced to three factors differing in intensity of effect: 1) pH of sea water (minor effect), 2) photolysis (additional minor effect), 3) trace-metal cation content of sea water (major effect), whereas that of xanthine could be traced only to photolysis.

These results cast doubt on previous reports of utilization of uric acid as N-source for phototrophic growth of marine planktonic algae in that the compound actually utilized may be degradation product(s) rather than the intact purine. Ecologically, the results signify that uric acid could undergo rapid chemical turnover, without biological intervention, in the marine environment.

Antia, N. J., and A. F. Landymore. 1974. Physiological and ecological significance of the chemical instability of uric acid and related purines in sea water and marine algal culture medium. J. Fish. Res. Board Can. 31: 1327–1335.

Nous avons évalué, par spectrophotométrie, l'instabilité chimique de l'acide urique et des bases puriques associées dans un milieu de culture de phytoplancton en eau de mer,

---

[1] Based in part on a M.Sc. thesis presented by A. F. L. to the University of British Columbia, Vancouver.

[2] Present address: Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1W5.

Printed in Canada (J3337)
Imprimé au Canada (J3337)

en mesurant les changements de concentration qui se produisent lors d'une incubation aseptique à environ 20 C sous éclairage blanc froid ou dans l'obscurité complète. Dans l'obscurité, l'acide urique montre une lente dégradation, rehaussée de beaucoup par l'éclairage. La xanthine accuse un faible degré de dégradation sous éclairage seulement, alors que l'adénine, la guanine et l'hypoxanthine semblent stables aux deux conditions d'essais. La dégradation de l'acide urique est attribuable à trois facteurs dont les effets diffèrent d'intensité: 1) pH de l'eau de mer (effet mineur), 2) photolyse (effet mineur additionnel), 3) teneur en cations de traces de métaux dans l'eau de mer (effet majeur). La dégradation de la xanthine ne peut être reliée qu'à la photolyse.

Ces résultats sont de nature à projeter un doute sur les rapports antérieurs concernant l'utilisation de l'acide urique comme source d'azote pour la croissance phototrophique d'algues planctoniques marines, puisque le composé utilisé peut, en fait, être un ou plusieurs produits de dégradation, plutôt que la purine intacte. Du point de vue écologique, les résultats signifient que l'acide urique pourrait subir un rapide écoulement chimique, sans intervention biologique, dans le milieu marin.

Received March 4, 1974
Accepted May 6, 1974

Reçu le 4 mars, 1974
Accepté le 6 mai, 1974

THE marine cryptomonad *Hemiselmis virescens* Droop was found to show good phototrophic growth on all the purines tested as the sole nitrogen source in the seawater culture medium used (Antia and Chorney 1968). When these tests were extended to xanthopterin and related pteridines as probable N-source for growth of the alga, we were surprised to find that several of these compounds underwent chemical modification and degradation in sea water. These changes were markedly enhanced by illumination from cool-white fluorescent lamps (Landymore 1971). This observation obliged us to verify the presumed stability of the previously tested purines in the seawater medium used for the algal growth tests. The results and subsequent developments of such an investigation made with uric acid, xanthine, hypoxanthine, guanine, and adenine are reported in this paper.

Materials and Methods

Open ocean water of salinity 32–33‰ and pH 8.0 (±0.1) was used throughout the study; this water was always filtered through membranes (0.45 μm pore size) before use. The algal culture medium was prepared by enriching this sea water with orthophosphate, silicate, vitamins (B_{12}, thiamine, biotin), trace-metal ions (Fe^{3+}, Mn^{2+}, Zn^{2+}, MoO_4^{2-}, Cu^{2+}, Co^{2+}) chelated with ethylenediamine tetraacetate (EDTA), and buffered with tris(hydroxymethyl)methyl amine to pH 7.8. The quantitative composition of the medium has been previously reported (Antia and Chorney 1968). Sodium phosphate buffer (0.05 M) was used for the comparative examination of the purines at pH 8.0 in the absence of sea water and the above enrichments.

Because of the difficult solubility of the purines in sea water, their concentrations were required to be sufficiently low (5–7 μM for uric acid, 20–50 μM for the other purines) to ensure complete dissolution. All solutions were prepared directly in the medium tested (culture medium, sea water, buffer) by heating until completely clear and were filter-sterilized (0.2μM pore-size Gelman Metricel filters in sterile disposable plastic units) after cooling. Suitable aliquots (50 ml) of these solutions were held aseptically in 125-ml capacity screwcapped Erlenmeyer flasks. A pair of such flasks was used for each purine studied, one for "incubation" in darkness and the other in light. Corresponding controls with the medium only (i.e. without purine) were always taken. All dark incubations were effected with total exclusion of light in a laboratory cupboard at ambient room temperature (22–25 C). All light incubations were effected in a plankton growth chamber maintained at 20–22 C and fitted with four parallel cool-white fluorescent lamps (in a horizontal plane above the test-flasks) providing continuous illumination of $6.0 \pm 0.3 \times 10^3$ erg/cm² s at the level of the test-flasks. An examination of the spectral energy distribution of the light from the fluorescent lamps indicated the following percent (of the total energy) contribution from the wavelength regions shown: 1.7%, 220–380 nm; 89.7%, 380–630 nm; 8.6%, 630–700 nm. The low-energy emission for wavelengths below 380 nm was shown to be lost from absorption by the glass walls of the test-flasks, so that the effective illumination acting on the test solutions was considered to lie in the range 380–700 nm.

Chemical changes of the purine solutions were monitored spectrophotometrically by periodically withdrawing standard aliquots (1 ml) from the test-flasks and reading their absorption spectra against the corresponding medium controls. Such monitoring was generally continued up to a maximum of 700 h (29 days). In general, the spectrum of each aliquot was scanned in the region 200–400 nm on a Beckman DB-G grating spectrophotometer, and the optical density (OD) at the absorption maximum (λmax) characteristic of each purine was then precisely read on a Beckman DU spectrometer. The test-aliquot was then acidified to pH near 1.0 by incorporating one drop of concentrated HCl into the sample in the cuvette and the absorption spectrum was again determined as before. The purine concentration (C mM) in test-solutions was computed from the optical densities measured at λmax for both pH values ($\text{OD}_{\text{pH }8.0}$, $\text{OD}_{\text{pH}1.0}$) in 1-cm light-path cuvettes using the equation:

$$C = \frac{10^3 \times \text{OD}_{\text{pH }8.0}}{\epsilon_{\text{pH }8.0}} = \frac{10^3 \times \text{OD}_{\text{pH }1.0}}{\epsilon_{\text{pH }1.0}}$$

Molar extinction coefficients ($\epsilon_{\text{pH }8.0}$, $\epsilon_{\text{pH }1.0}$) at pH values near 8.0 and 1.0, respectively, were carefully determined for all the purines tested in both sea water and appropriate buffers without sea water; the spectral region 200–225 nm was ignored and effects of small pH differences (± 0.2 unit) were discounted (see Table 1). In all cases, the λmax showing the highest extinction coefficient was chosen for measuring concentrations. The computed concentrations were plotted against incubation time to obtain purine reaction rate in the appropriate medium. Following standard chemical nomenclature, the purine reaction kinetics were designated zero or first order, respectively, according to the linear or logarithmic nature of the concentration-change rate observed. Where a common denominator was required for rate comparison of the different purines, first-order reaction rates have been mathematically approximated to zero-order rates of lower precision value; these rates are valid only for the purine concentration initially present but provide an order of magnitude useful for the comparisons required.

Care was taken throughout these studies to maintain sterile conditions and the test solutions were routinely checked for microbial contamination by inoculating 1–2 drops into standard aliquots (2.5 ml) of STP or ST_3 media of Tatewaki and Provasoli (1964) and incubating for 3 wk in darkness at room temperature.

Results and Conclusions

All the purines showed no discernible shift in their characteristic absorption maxima from the incubations in light or darkness, but progressive changes were observed in the optical density at these maxima. These changes will be considered separately as follows.

INCUBATIONS IN DARKNESS

Adenine, guanine, hypoxanthine, and xanthine showed minute but measurable rates of OD increase over part or whole of the total period studied in the algal culture medium (Table 2, Fig. 1). As this increase persisted even after acidification of the test-samples, it does not appear to be due to slow complex-formation of the purines with one or more

TABLE 1. Molar extinction coefficients at characteristic absorption maxima[a] of purines in sea water and buffer solutions without sea water.

	Seawater medium[b]			Buffer solution[c]		
Purine	pH	λmax (*nm*)	ε (×10³) (*liter mole*$^{-1}$ *cm*$^{-1}$)	pH	λmax (*nm*)	ε (×10³) (*liter mole*$^{-1}$ *cm*$^{-1}$)
Adenine	7.8	260	11.9	8.0	260	11.9
	1.0	263	11.7	1.0	263	11.7
Guanine	7.8	246	11.5	8.0	246	9.6
		276	7.5		276	8.1
	1.0	248	15.1	1.0	247	10.1
		273	7.8		274	7.4
Hypoxanthine	7.8	249	12.8	8.0	250	10.0
	1.0	249	13.2	1.0	249	9.9
Uric acid	7.8	236	10.1	8.0	236	7.7
		292	12.6		292	10.0
	1.0	230	9.8	1.0	230	8.0
		285	12.3		285	11.1
Xanthine	7.8	243	7.8	8.0	244	7.3
		272	9.8		274	8.4
	1.0	227	7.1	1.0	228	6.2
		262	10.4		262	8.8

[a] Only the spectral region 225–300 nm is considered here.

[b] Algal culture medium buffered at pH 7.8 (±0.2); the two pH values shown correspond to those of the medium before and after acidification (see text).

[c] Purine solutions in 0.05 M sodium phosphate buffer (pH 8.0) or HCl–KCl buffer (pH 1.0).

TABLE 2. Reaction rates of purines in marine algal culture medium from incubation in darkness and light.

Purine	Type of OD change at principal λmax		Reaction kinetics[a] Order		Velocity[b] (μM/100 h)	
	Dark	Light	Dark	Light	Dark	Light
Adenine	increase		zero		0.09	0.08
Guanine	increase		zero		0.07	0.17
Hypoxanthine	increase		ND	zero	ND	0.13
Uric acid	decrease		zero	first	0.76	12.0[c]
Xanthine	increase	decrease	ND	zero	ND	0.34

[a] Symbols ND denote that these parameters could not be adequately determined; for other details see text.

[b] Calculated on the basis that OD increase or decrease reflect, respectively, the equivalent production of a modified purine or the degradation of the original purine by zero-order kinetics.

[c] This value is a proximate zero-order equivalent of the first-order kinetics constant (3.15×10^{-3} h^{-1}) actually obtained.

components of the seawater medium, as such complexes would be expected to dissociate at pH 1.0. We do not know the reason for this OD increase, but its low magnitude (less than 1% of total purine affected per 100 h), coupled with the permanence of the overall spectral profile, leads us to infer that these purines are not significantly affected by long-term residence in seawater medium.

By contrast, uric acid showed a steady OD decrease with zero-order reaction kinetics (Table 2, Fig. 1). This decrease appeared to be uniform throughout the absorption spectrum, which indicated continuous decomposition of the uric acid without recognizable accumulation of purine-type intermediates (Fig. 2). As the uric acid concentration dropped to low levels, there emerged a trend of increase in the absorption at the secondary maximum (236 nm) relative to the primary maximum (292 nm), suggesting the ultimate accumulation of products absorbing with low intensity in the far UV-region. The rate of reaction was sufficient to account for complete decomposition of 1 μM uric acid in 133 h or 5.5 days.

INCUBATIONS IN LIGHT

Adenine, guanine, and hypoxanthine showed minute OD increases (without change in the overall spectrum) similar to those obtained in darkness and there was no clear indication of chemical reaction or decomposition (Table 2, Fig. 1 and 3). However, xanthine showed continuous OD decrease indicative of chemical decomposition by a slow zero-order reaction; the estimated rate could account for complete decomposition of 1 μM xanthine in 300 h or 12.5 days. Uric acid showed rapid decomposition with first-order reaction kinetics and at an initial rate about 15 times that obtained in darkness (Table 2, Fig. 1). With both these purines showing decomposition, there was no spectroscopically recognizable indication of purine-type products (Fig. 2 and 3). No attempt was made to identify further degradation products.

URIC ACID DECOMPOSITION IN NATURAL SEA WATER AND BUFFER OF pH 8.0

The above results obtained with the algal culture medium suggested that the observed decompositions may be caused by the salts or ionic strength of the seawater base. The decomposition of uric acid in natural sea water alone was compared directly with that in a buffer at pH 8.0. The results (Table 3) showed that 1) the pH alone causes a small degree of uric acid decomposition in darkness, which reaction is roughly doubled by light, but 2) the sea water causes 90–180 times the decomposition obtained in the buffer of comparable pH, with surprisingly little effect from light. The comparison of these results with those obtained from the algal culture medium (see Table 3) indicated that 1) the uric acid reaction rate in the culture medium was roughly comparable to that in the buffer for dark incubations but was at least tenfold greater under light, 2) the decomposition in natural sea water was at least 100-fold greater under darkness and about sevenfold greater under light than in the culture medium.

It was inferred from these observations with pH as a common denominator that a component of sea water other than its major ions was responsible for the increased decomposition of uric acid, the effectiveness of which component was repressed in the culture medium under darkness but was derepressed under light. A consideration of the nutrient additives in the culture medium suggested that this seawater component may be the trace-metal cations which are free in the natural sea water and bound by EDTA in the culture medium. When free in the sea water, they are present in adequate concentration to cause maximal effect on uric acid with little or no effect from light, but when bound by EDTA their action is repressed in darkness. As light is known to release at least some types of these ions by photoreductive decomposition of their EDTA-complexes (Hill-Cottingham 1955; Jones and Long 1952), their effectiveness toward uric acid is expected to be derepressed by the action of light on the culture medium. The photolysis of these complexes also causes decomposition of the EDTA moiety so that a considerable excess of EDTA must be initially present to repeatedly recapture the liberated ions and maintain them in bound form. The EDTA concentration in the culture medium (21.8 μM) is known to be in excess of that required to bind the trace-metal ion mixture (18.2 μM) added as nutrient enrichment and is expected to additionally bind the corresponding free ions present in the seawater base of the medium. Standard determinations by the methods of Strickland and Parsons (1972) have shown concentrations of 0.01 μM Fe^{2+} and 0.18 μM Cu^{2+} in the sea water used after filtration. However, this excess EDTA concentration in the culture medium could be insufficient to compensate for the loss from photolysis.

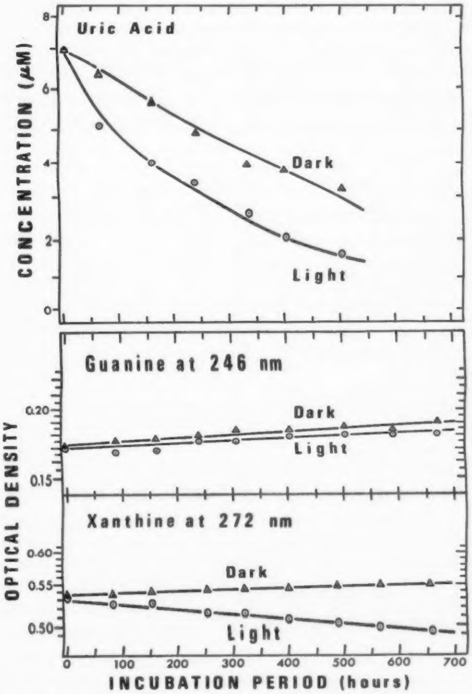


FIG. 1. Changes with incubation period of uric acid concentration, absorbances of xanthine and guanine at their characteristic λmax. All incubations were effected in the seawater culture medium; the absorbances shown were measured in this medium before acidification (see text).

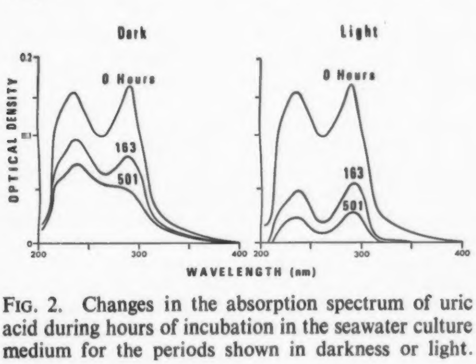


FIG. 2. Changes in the absorption spectrum of uric acid during hours of incubation in the seawater culture medium for the periods shown in darkness or light.

These inferences were verified by studying the uric acid reactions with an excess of EDTA added to the natural sea water and a comparable excess added to the chelator concentration already present in the culture medium. In both cases (Table 3), the decomposition rate, whether in light or darkness, was dramatically reduced, by the presence of this excess EDTA, to levels approaching those obtained with the buffer alone. It was thus established that 1) the accelerated decomposition of uric acid in the natural sea water independent of light and that obtained in the culture medium in presence of light were due to the action of free trace-metal ions, 2) the amount of EDTA normally added to the culture medium in conjunction with the trace-metal ion enrichment was insufficient to offset the photolytic liberation of these ions from binding by chelation.

In view of these findings, it was important to check whether the behavior of the other purines in seawater medium might also be affected by trace-metal ions. Such tests showed that the light-induced decomposition rate of xanthine in natural sea water

(0.36 μM/100 h, comparable to that obtained in the culture medium; see Table 2) was surprisingly doubled (to 0.81 μM/100 h) from the presence of EDTA (5.0 μM), suggesting an inhibitory effect of trace-metal ions on the photolysis of xanthine. The darkness stability of xanthine noted with the culture medium was confirmed with natural sea water and there was no effect from the presence of EDTA in the latter. Similarly, hypoxanthine showed complete stability in natural sea water under light or darkness and there was no effect from EDTA. It was thus concluded that the seawater trace-metal ions accelerated the decomposition of uric acid only among the purines tested. The opposite (inhibitory) effect of these ions on the photolysis of xanthine appears to be of minor magnitude but further studies are needed to assess its significance.

Apart from these effects of trace-metal ions, it is also concluded from our results that 1) both uric acid and xanthine undergo a significant degree of photolytic degradation in sea water, and 2) uric acid (but not xanthine) is also degraded slowly by

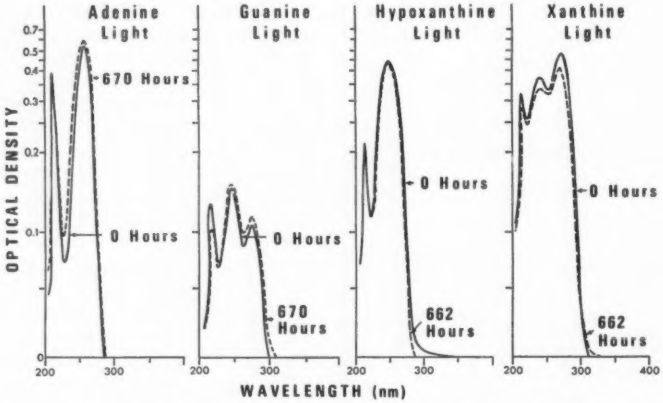


FIG. 3. Changes in the absorption spectra of adenine, guanine, hypoxanthine, and xanthine during incubation in the seawater culture medium for the hours shown in light.

TABLE 3. Decomposition rates of uric acid in different media during incubation in darkness or light.

	Reaction kinetics			
	Order		Velocity[a] (μM/100 h)	
Medium	Dark	Light	Dark	Light
Na-phosphate buffer pH (8.0, 0.05 M)	zero		0.45	1.04
Sea water (natural)	first		83.5(0.021)	90.0(0.022)
Sea water + EDTA[b]	zero		0.72	1.49
Algal culture medium	zero	first	0.76	12.0(0.003)
Culture medium + EDTA[b]	zero		0.65	1.34

[a] All values are expressed in terms of zero-order kinetics; values in parentheses denote the corresponding first-order rate constants (h^{-1}).

[b] This addition was calculated to give a final concentration of 5.0 μM in the sea water, and an increment by 5.0 μM of the EDTA concentration (21.8 μM) already present in the culture medium.

the pH alone, whereas 3) adenine, guanine, and hypoxanthine are stable in sea water under all conditions tested.

Discussion

Using a similar method of measurement, Birdsey (1962) observed a considerable rate of decrease, from incubation in light, of the concentration of uric acid dissolved in an artificial-seawater algal culture medium, but this was attributed to slow precipitation of the difficultly soluble purine from the medium. This explanation may be partly true since the uric acid concentration used (0.35 mM) was near saturation. For this reason, we took particular care to use subsaturating concentrations of all purines in this study. Even with such concentrations (0.05, 0.022 mM), initially clear solutions of uric acid showed gradual microprecipitation after long delays; eventually the low concentration range 0.005–0.007 mM was chosen to ensure permanent dissolution of uric acid.

Our conclusion that uric acid degradation in sea water is accelerated by free trace-metal ions is based entirely on evidence obtained from EDTA inhibition of such degradation. The possibility must be considered that this EDTA effect may be exercised on trace-metal ions bound with uric-acid oxidizing enzymes or with natural organic chelators (Barber and Ryther 1969; Lewis et al. 1971) in the sea water. In both cases, EDTA, with its generally stronger chelation capacity, could displace the trace-metal ions from such natural binding, and its net effect would be the same as if these ions had existed free in the sea water. The probability of enzyme involvement in uric acid degradation appears unlikely in view of the heat treatment given to the seawater and culture medium to dissolve the purines, unless such enzymes happen to be exceptionally heat-stable. The enzyme uricase effecting oxidative degradation of uric acid to allantoin is believed to require Cu^{2+} for activity (Mahler 1963); there appears to be no information in the literature on its heat stability or its occurrence in sea water. On the other hand, several lines of evidence indicate the occurrence in sea water of natural chelators binding Cu^{2+} (Williams 1969; Nielsen and Wium-Andersen 1970), Fe^{2+} (Lewin and Chen 1973), Mn^{2+} and Zn^{2+} (Rona et al. 1962); these chelators are suggested to be composed of polypeptides, lipids, or humic substances (Prakash et al. 1973) and most of them are expected to be displaced from ion-complexes by EDTA. As with the previous researchers, we are unable to distinguish the naturally-chelated from truly free ions in the effects obtained from the so-called free trace-metal ions on uric acid decomposition in sea water.

Our findings on the photolytic degradation of uric acid in the culture medium cast doubt on previous reports of the utilization by marine algae of this purine as sole nitrogen source for phototrophic growth (Antia and Chorney 1968; Gibor 1956; Droop 1955; Guillard 1963; Van Baalen and Marler 1963), in that the algae may be using degradation products as the N-source rather than the intact purine. Regarding the trace-metal ion effect, the degree of this doubt will depend on the excess proportion of EDTA (or artificial chelator) relative to these ions incorporated in the various culture media used. Even with the total control of this ionic effect, it appears that the photolytic degradation rate of uric acid could provide sufficient artifact nitrogen (estimated at about 25 μg-at/liter produced in 20 days) to support modest growth of an alga in culture tests, particularly in the case of species showing slow growth or requiring substantial periods of adaptation. Unfortunately we have not been able to identify the uric acid degradation products, so that the capability of these products to support algal growth remains open to question. The proven stability of adenine, guanine, and hypoxanthine in sea water leads us to recommend them as unquestionably intact purine sources of nitrogen for marine algal growth studies. If these studies are intended to establish algal capacity to use the purine-skeleton nitrogen atoms as sole N-source for growth, we would consider hypoxanthine as the prime logical candidate for examination, as it not only lacks the exocyclic amino groups present in adenine and guanine but it also offers scope for testing at considerably higher concentration by virtue of its greater solubility than the other purines.

Ecologically speaking, our results leave no doubt that dissolved uric acid would undergo rapid chemical degradation or turnover, without biological intervention, in the marine environment. With the trace-metal ion content found in our seawater sample, we estimate the half-life of uric acid at about 31–33 h in sea water at 20 C irrespective of sunlight. The effect of temperature remains unknown. Although considerably less, a significant chemical turnover of xanthine in marine regions is also anticipated by our results; this turnover will be entirely governed by the intensity of sunlight available. In this connection, it is pointed out that the estimated radiant energy and spectral distribution from the light source used in the present study fall within the range of solar radiation normally penetrating oceanic waters, at least in the UV and visible regions; the greater intensity of red and infrared radiation from sunlight is known to be lost from absorption at the water surface (Strickland 1958). The occurrence of purines in the marine environment is to be expected from present knowledge of their production by marine fauna. Considerable amounts of guanine and hypoxanthine have been found in fish skin and scales (Fujii 1969), whereas uric acid and guanine are common, sometimes predominant, constituents of excrements of birds and mammals, and they are often accompanied by lesser amounts of adenine, hypoxanthine, and xanthine (Bendich 1955). Coastal waters may be expected to receive considerable purine enrichment from land drainage and sewage. It is therefore surprising that they have been scarcely reported in sea water; only adenine has been positively detected (Litchfield and Hood 1966). Presumably the low solubility and rapid degradation of uric acid militate against its isolation and identification by oceanographers. The low solubility does suggest uric acid precipitation from sea water and accumulation in solid phase, but the available geochemical evidence indicates no such substantial purine deposition in the oceanic environment apart from the well-known guano deposits localized on some rocky shores and islands. We therefore surmise that the solid-phase uric acid is in continuous equilibrium with its dissolved phase and undergoes continual chemical degradation via the latter phase; additional microbial degradation may occur in both phases. Regarding the turnover of the stable purines, there is some evidence of metabolic degradation of adenine, guanine, and hypoxanthine by marine phytoplankters (Antia and Chorney 1968; N. J. Antia unpublished data); other marine microorganisms have not been tested in this respect.

The observed order of stability of these purines in sea water is in accord with that predicted by chemical considerations which indicate gain in molecular reactivity from increase in the number of hydroxyl substituents on the purine ring skeleton (Albert and Brown 1954). The photolysis of purines from UV-irradiation is well-known (Shugar 1960) but that from white-light illumination has not been reported. It is possible that the residual low-energy irradiation in the 220–380 nm region emanating from our light source may have caused the photolysis of xanthine and uric acid observed in the absence of trace-metal ion effects. Uric acid is known to be oxidized by air in strongly alkaline solutions and this oxidation is reported to be accelerated by low Cu^{2+} concentration (Stahl 1969). A similar oxidative mechanism may be involved in the uric acid degradation by sea water, as air was not excluded in our tests.

Acknowledgments

We are grateful to Dr P. A. Larkin, Department of Zoology, University of B.C., for his encouragement of this investigation and for arranging financial support of A. F. L. through the Fisheries and Marine Service.

Albert, A., and D. J. Brown. 1954. Purine studies. Part I. Stability to acid and alkali, solubility, ionization. Comparison with pteridines. J. Chem. Soc. 1954: 2060–2071.

Antia, N. J., and V. Chorney. 1968. Nature of the nitrogen compounds supporting phototrophic growth of the marine cryptomonad *Hemiselmis virescens*. J. Protozool. 15: 198–201.

Barber, R. T., and J. H. Ryther. 1969. Organic chelators: Factors affecting primary production in the Cromwell Current upwelling. J. Exp. Mar. Biol. Ecol. 3: 191–199.

Bendich, A. 1955. Chemistry of purines and pyrimidines, p. 81–136. *In* E. Chargaff and J. N. Davidson [ed.] The nucleic acids, chemistry and biology. Vol. 1. Academic Press, New York, N.Y. 692 p.

Birdsey, E. C. 1962. The nitrogen utilization and metabolism of unicellular algae. M.A. Thesis. Stanford University, Stanford, Calif. 114 p.

Droop, M. R. 1955. Some new supra-littoral protista. J. Mar. Biol. Ass. U.K. 34: 233–245.

Fujii, R. 1969. Chromatophores and pigments, p. 307–353. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. 3. Academic Press, New York and London. 485 p.

Gibor, A. 1956. The culture of brine algae. Biol. Bull. 111: 223–229.

Guillard, R. R. L. 1963. Organic sources of nitrogen for marine centric diatoms, p. 93–104. *In* C. H. Oppenheimer [ed.] Symposium on marine microbiology. C. C. Thomas, Springfield, Ill. 769 p.

Hill-Cottingham, D. G. 1955. Photosensitivity of iron chelates. Nature 175: 347–348.

Jones, S. S., and F. A. Long. 1952. Complex ions from iron and ethylenediamine tetraacetate: general properties and radioactive exchange. J. Phys. Chem. 56: 25–33.

Landymore, A. F. 1971. The chemical (non-biological) and photolytic transformations of pteridines and purines effected by the salts of seawater, and their ecological significance. M.Sc. Thesis. Univ. British Columbia, Vancouver, B.C. 152 p.

Lewin, J., and C.-H. Chen. 1973. Changes in the concentration of soluble and particulate iron in seawater enclosed in containers. Limnol. Oceanogr. 18: 590–596.

Lewis, A. G., A. Ramnarine, and M. S. Evans. 1971. Natural chelators — an indication of activity with the calanoid copepod *Euchaeta japonica*. Mar. Biol. 11: 1–4.

Litchfield, C. D., and D. W. Hood. 1966. Microbiological assay for organic compounds in seawater II. Distribution of adenine, uracil, and threonine. Appl. Microbiol. 14: 145–151.

Mahler, H. R. 1963. Uricase, p. 285–296. *In* P. D. Boyer, H. Lardy, and K. Myrbäck [ed.] The enzymes. 2nd ed. (revised). Vol. 8. Academic Press, New York and London. 484 p.

NIELSEN, E. S., AND S. WIUM-ANDERSEN. 1970. Copper ions as poison in the sea and in freshwater. Mar. Biol. 6: 93–97.

PRAKASH, A., M. A. RASHID, A. JENSEN, AND D. V. SUBBA-RAO. 1973. Influence of humic substances on the growth of marine phytoplankton: diatoms. Limnol. Oceanogr. 18: 516–524.

RONA, E., D. W. HOOD, L. MUSE, AND B. BUGLIO. 1962. Activation analysis of manganese and zinc in seawater. Limnol. Oceanogr. 7: 201–206.

SHUGAR, D. 1960. Photochemistry of nucleic acids and their constituents, p. 39–104. *In* E. Chargaff and J. N. Davidson [ed.] The nucleic acids. Vol. 3. Academic Press, New York and London. 588 p.

STAHL, P. H. 1969. The product of the air oxidation of uric acid. An intermediate formed in the presence of dimethylamine. Biochemistry 8: 733–736.

STRICKLAND, J. D. H. 1958. Solar radiation penetrating the ocean. A review of requirements, data and methods of measurement, with particular reference to photosynthetic productivity. J. Fish. Res. Board Can. 15: 453–493.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. 2nd ed. Bull. Fish. Res. Board Can. 167: 310 p.

TATEWAKI, M., AND L. PROVASOLI. 1964. Vitamin requirements of three species of *Antithamnion.* Bot. Mar. 6: 193–203.

VAN BAALEN, C., AND J. E. MARLER. 1963. Characteristics of marine blue-green algae with uric acid as nitrogen source. J. Gen. Microbiol. 32: 457–463.

WILLIAMS, P. M. 1969. The association of copper with dissolved organic matter in seawater. Limnol. Oceanogr. 14: 156–158.

Descriptions of Copepodite Stages of *Chiridius gracilis* Farran 1908 (Crustacea: Copepoda)[1]

DELPHINE C. MACLELLAN

Marine Sciences Centre, McGill University, Montreal, Que.

AND CHANG-TAI SHIH

Canadian Oceanographic Identification Centre
National Museum of Natural Sciences, Ottawa, Ont. K1A 0M8

MACLELLAN, D. C., AND C.-T. SHIH. 1974. Descriptions of copepodite stages of *Chiridius gracilis* Farran 1908 (Crustacea: Copepoda). J. Fish. Res. Board Can. 31: 1337–1349.

On the basis of specimens taken from plankton samples, all copepodite stages of *Chiridius gracilis* are described and illustrated. The first thoracic somite is fused with the cephalosome in adults, but an incomplete suture exists between these body parts in copepodites IV and V. The metasomal process, a prominent character of this species, appears as a small blunt process as early as copepodite I and becomes fully developed by copepodite IV. Segmentation of mouthparts is complete by copepodite IV but full setation is not acquired until the adult stage. The mandible, maxillule, and maxilla degenerate in the adult male. Segmentation and setation of swimming legs are complete in copepodite V in the female and in the adult male.

MACLELLAN, D. C., AND C.-T. SHIH. 1974. Descriptions of copepodite stages of *Chiridius gracilis* Farran 1908 (Crustacea: Copepoda). J. Fish. Res. Board Can. 31: 1337–1349.

A partir de spécimens provenant d'échantillons de plancton, nous décrivons et figurons tous les stades copépodites de *Chiridius gracilis*. Le premier somite thoracique est fusionné avec le céphalosome chez les adultes, mais il existe une suture incomplète entre ces parties du corps chez les copépodites IV et V. Le procès métasomique, un caractère proéminent de cette espèce, apparaît sous forme d'un petit procès mousse dès le copépodite I et atteint son plein développement au copépodite IV. La segmentation des pièces buccales est complète au stade copépodite IV, mais les soies ne sont complètement développées qu'au stade adulte. Les mandibule, maxillule et maxille se dégénèrent chez le mâle adulte. La segmentation et la formation des soies des pattes natatoires sont complètes au stade copépodite V chez la femelle et chez le mâle adulte.

Received November 6, 1973
Accepted April 9, 1974

Reçu le 6 novembre, 1973
Accepté le 9 avril, 1974

Chiridius gracilis, calanoid copepod occurring in deep waters of temperate to tropical regions of all oceans, was recorded first as *C. poppei* by Farran (1905) but redescribed later as a new species from an adult female from slope waters off the west coast of Ireland (Farran 1908). Vervoort (1957) and Tanaka and Omori (1970) summarized other records which, with a few exceptions, were of adult females. Farran (1929) recorded one copepodite stage IV male without describing it. Tanaka (1937) briefly described a copepodite stage V male of *Chiridius* sp. from Japanese waters and thought it might be a male of *C. gracilis*. In 1957 he described a mature male from the same area. Farran (1908) and Tanaka (1957) published certain characteristic features of the adult stages but their descriptions were incomplete. The morphology of other copepodite stages of this species has previously been practically unknown.

This paper describes all copepodite stages of *C. gracilis* on the basis of 1406 specimens collected from 11 stations in the Gulf of St. Lawrence (see map). For comparison we also obtained several collections of this species from the Atlantic and Pacific oceans. In the western North Atlantic the species was previously reported by Grice (1963)

---

[1] Contribution to the International Biological Program CCIBP No. 216.

Printed in Canada (J3218)
Imprimé au Canada (J3218)

and Park (1970). The present records extend its known northern range in the northwestern Atlantic.

Methods

Total lengths, prosome lengths, and urosome lengths were measured with a Wild M5 microscope equipped with a measuring eyepiece. All available specimens of copepodites I–III were measured. From copepodite IV to adults, the means of total lengths, prosome lengths and ratios of prosome to urosome and their 95% confidence limits were based on the measurements of 30 specimens.

Specimens were dissected in glycerine. Appendages were placed in a drop of glycerine on a cover slip, which was then inverted over the cavity of a well slide (Humes and Gooding 1964). All drawings were made with a Wild M20 microscope with drawing tube.

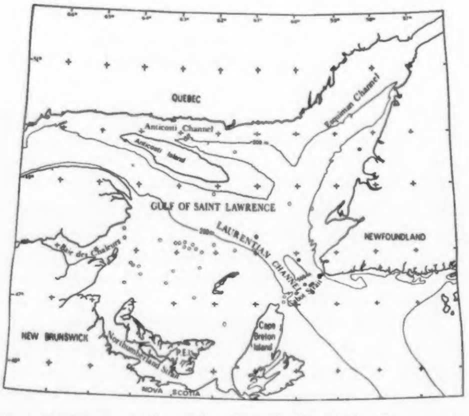
Morphological terms mainly follow those of Fleminger (1967).

Copepodite I

(FIG. 1–9)

Total length (excluding caudal setae) 0.56–0.60 mm; prosome 0.47–0.49 mm. First thoracic somite fused with cephalosome. Metasome 1-segmented, with rounded posterior margin bearing a blunt process on each posterior lateral corner. Urosome 2-segmented. Second urosomal somite twice the length of first. Ratio of prosome to urosome about 4.

Antennule 10-segmented. Antenna with 2-segmented protopod, 2-segmented endopod, and 7-segmented exopod. Mandible with 2-segmented protopod, 2-segmented endopod, and 5-segmented exopod; mandibular gnathobase with seven teeth. Maxillule with first outer lobe; exopod 1-segmented, larger than 2-segmented endopod; first to third inner lobes present. Maxilla with five lobes; segmentation of endopod invisible. Spinules lacking on surface of first inner lobe of maxillule and first to fourth lobes of maxilla. Endopod of maxilliped 2-segmented.



Zooplankton stations in the Gulf of St. Lawrence. ●, stations where *Chiridius gracilis* was taken; ○, stations where *C. gracilis* was not taken.

P1 and P2 with 2-segmented protopod and 1-segmented exopod and endopod. P1 protopod naked; exopod with three lateral spines, one smooth terminal spine, and three medial setae; endopod with five setae. P2 protopod naked; exopod with three lateral spines, one elongate serrate terminal spine, and three medial setae; endopod with five setae.

Occurrence: May, July, and August.

Copepodite II

(FIG. 10–19)

Total length 0.85–0.92 mm; prosome 0.69–0.76 mm. Number and relative lengths of urosomal segments same as in copepodite I. Ratio of prosome to urosome 4.0–4.5.

Antennule 17-segmented. Maxilla with a few spinules on first and second lobes; endopod 3-segmented. Maxilliped with 3-segmented endopod.

P1, B2 with a spine at distal end of lateral margin, and a straight comb seta near distal end of medial margin; endopod S1 with small tubercle with spinules on lateral margin. P2, B1 with medial seta; endopod 1-segmented with six setae; exopod 2-segmented, S1 with one spine, S2 with two lateral spines, one terminal spine, and four medial setae. P3, B1 without medial seta; endopod 1-segmented with six setae; exopod 1-segmented with three lateral spines, one terminal spine, and three medial setae.

Occurrence: April, May, and July–September.

Copepodite III

(FIG. 20–30)

Total length 1.16–1.28 mm; prosome 0.96–1.02 mm. Metasome 3-segmented. Ratio of prosome to urosome as before.

Antennule 22-segmented. Spinules on surface of first inner lobe of maxillule and first to fourth lobes of maxilla. Endopod of maxilliped 4-segmented.

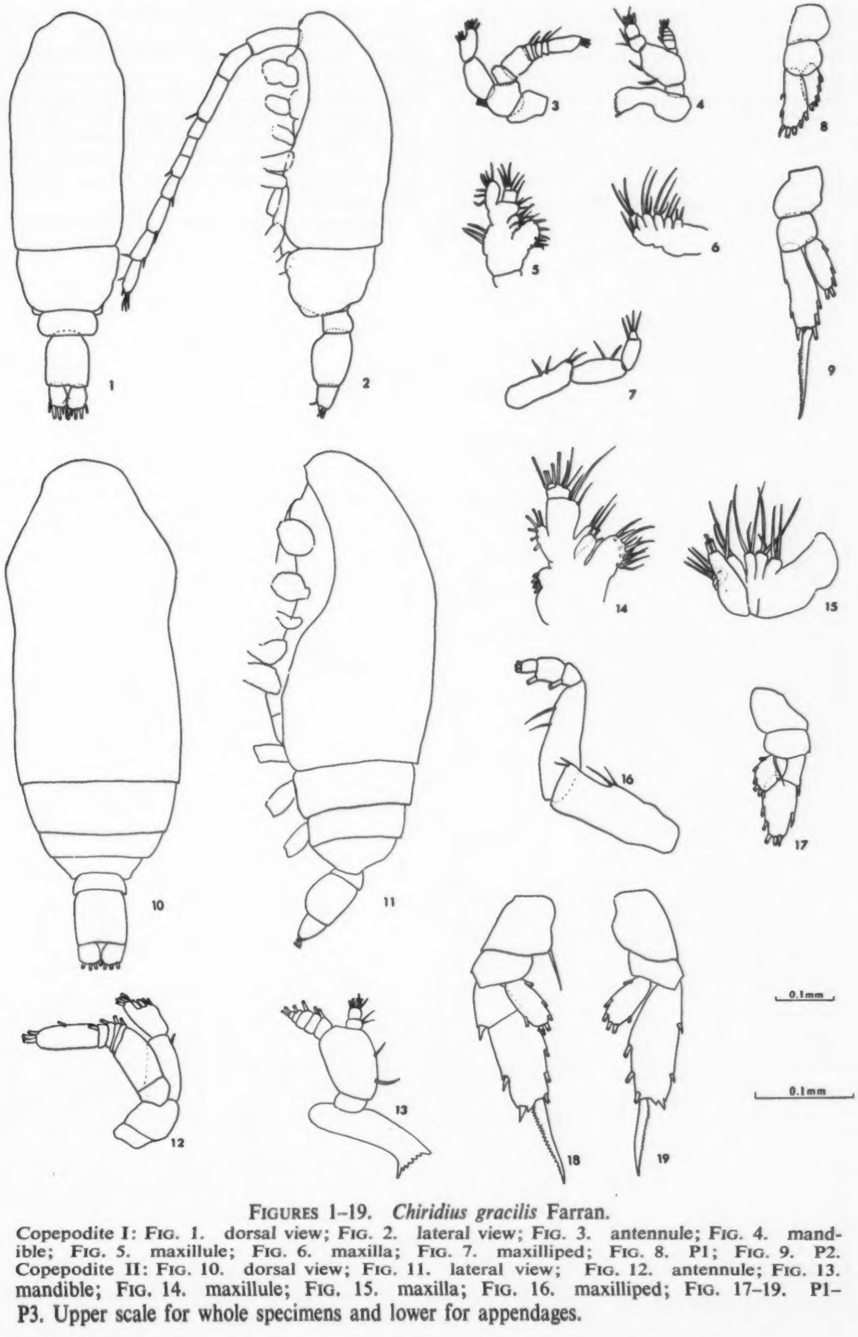
P2 exopod with one spine and one seta in S1 and three lateral spines, one terminal spine, and five medial setae in S2. P3 exopod 2-segmented, with one spine in S1, and with two lateral spines, one terminal spine, and four medial setae in S2. P4 endopod 1-segmented, with six setae; exopod 1-segmented, with three lateral spines, one terminal spine, and three medial setae.

Occurrence: May to September.

Copepodite IV

(FIG. 31–42, male; FIG. 43–53, female)

Sexes distinguishable on appearance of P5 in male. Total length of male 1.66 ± 0.11 mm (95% confidence limits), female 1.61 ± 0.11 mm; prosome 1.29 ± 0.08 mm in male and 1.25 ± 0.10 mm in female.



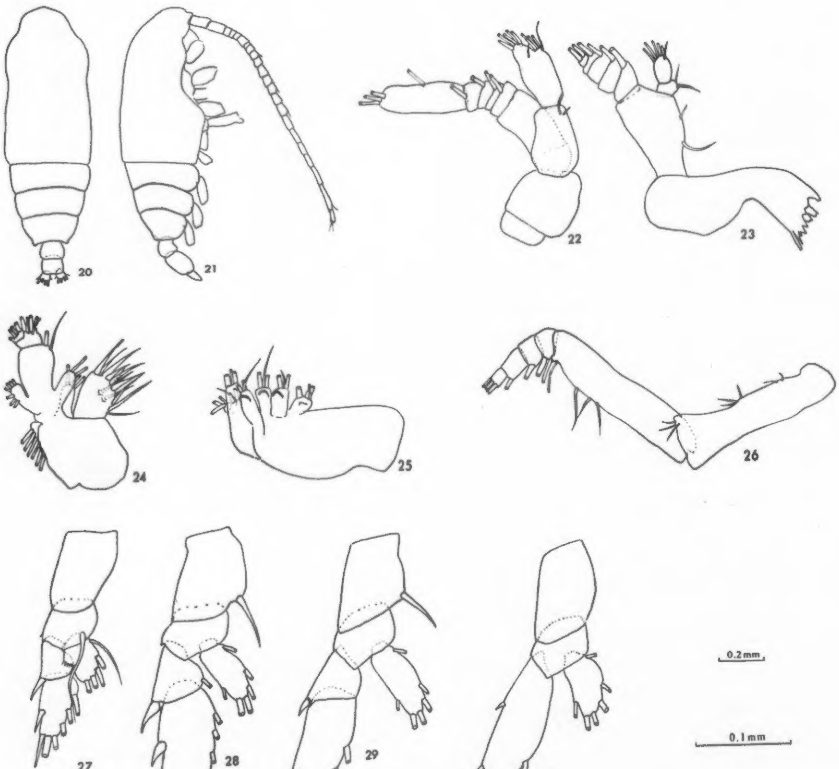
FIGURES 1–19. *Chiridius gracilis* Farran.
Copepodite I: FIG. 1. dorsal view; FIG. 2. lateral view; FIG. 3. antennule; FIG. 4. mandible; FIG. 5. maxillule; FIG. 6. maxilla; FIG. 7. maxilliped; FIG. 8. P1; FIG. 9. P2. Copepodite II: FIG. 10. dorsal view; FIG. 11. lateral view; FIG. 12. antennule; FIG. 13. mandible; FIG. 14. maxillule; FIG. 15. maxilla; FIG. 16. maxilliped; FIG. 17–19. P1–P3. Upper scale for whole specimens and lower for appendages.

Metasome with three free segments, last segment formed by the fusion of fourth and fifth thoracic somites. Suture between cephalosome and first thoracic somite, obscure in previous stages, now relatively distinct but incomplete. Metasomal processes, which are characteristic of this species, well developed. Ratio of prosome to urosome 4.31 ± 0.27 in male and 4.21 ± 0.28 in female.

Antennules 23-jointed, with fusions at 8th–9th and 24th–25th segments. Maxilliped with 5-segmented endopod.

P1 and P2 similar to those of previous stage but marginal hairs on legs more abundant. P3 and P4 exopod 2-segmented, with one spine and one seta in S1 and with three lateral spines, one terminal spine and five medial setae in S2. P5, which appears only in male, uniramous, 2-segmented, and nearly symmetrical. Second segment of left leg slightly narrower and longer than right; each with three spines, one at middle of lateral margin and two at distal margin, medial distal being longest.

Occurrence: January, and May–September.



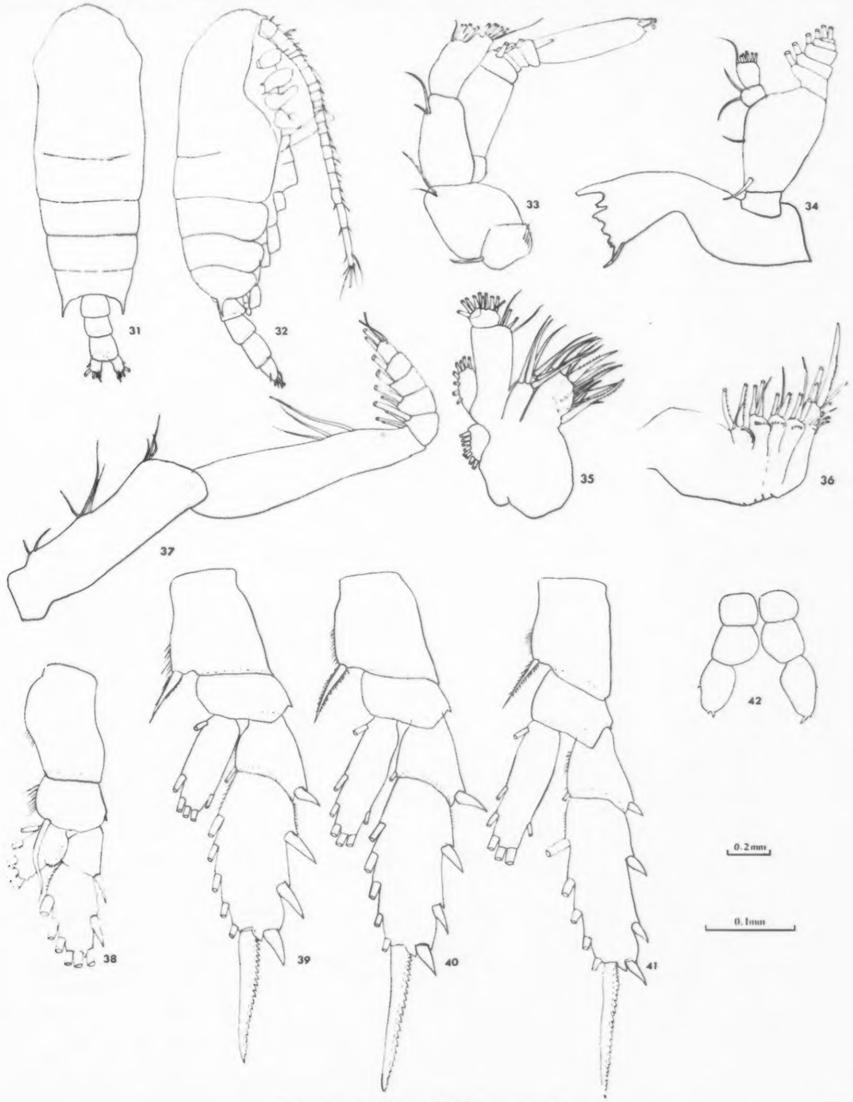
FIGURES 20–30. *Chiridius gracilis*.

Copepodite III: FIG. 20. dorsal view; FIG. 21. lateral view; FIG. 22. antennule; FIG. 23. mandible; FIG. 24. maxillule; FIG. 25. maxilla; FIG. 26. maxilliped; FIG. 27–30. P1–P4. Scales as for Fig. 1–19.

Copepodite V

(Fig. 54–65, male; Fig. 66–76, female)

Total length of male 2.25 ± 0.21 mm, female 2.21 ± 0.12 mm; prosome 1.71 ± 0.10 mm in male and 1.67 ± 0.10 mm in female. Males and females similar in general appearance as well as in anatomy of appendages. Incomplete suture between cephalosome and first thoracic somite present. Urosome 4-segmented. Ratio of prosome to urosome 3.87 ± 0.30 in male and 3.78 ± 0.15 in female.

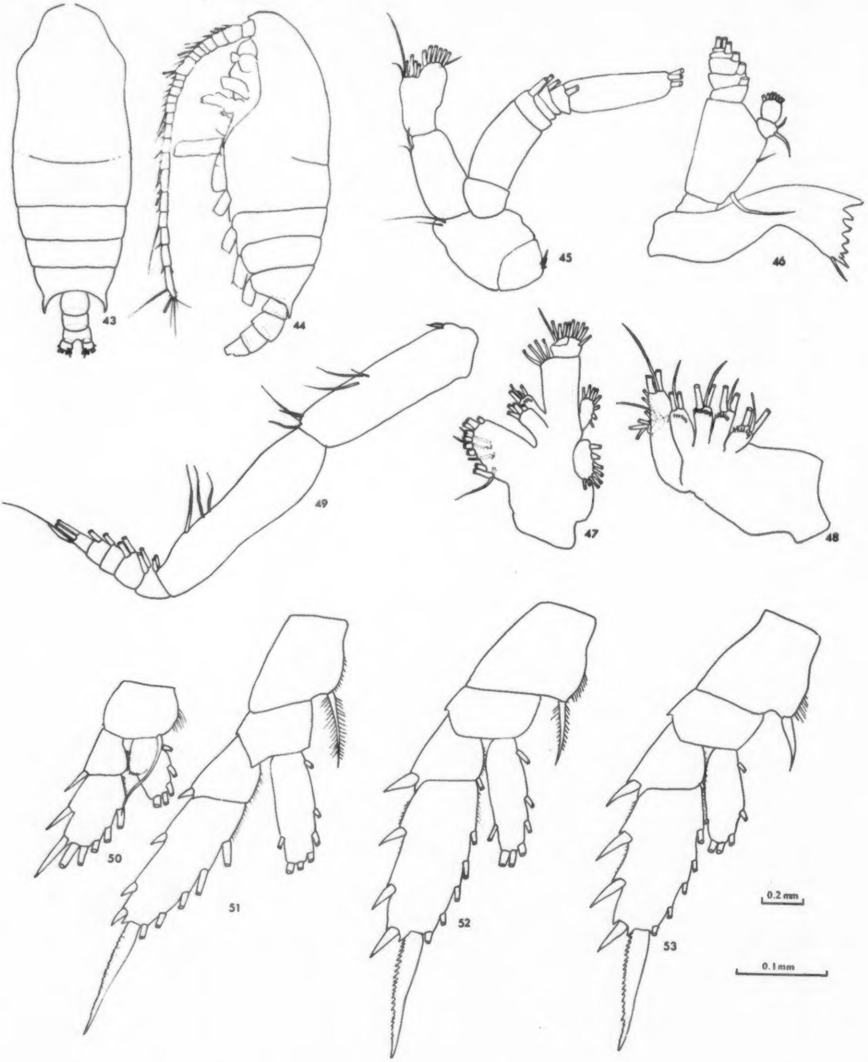


Figures 31–42. *Chiridius gracilis.*

Copepodite IV, male: Fig. 31. dorsal view; Fig. 32. lateral view; Fig. 33. antennule; Fig. 34. mandible; Fig. 35. maxillule; Fig. 36. maxilla; Fig. 37. maxilliped; Fig. 38–42. P1–P5. Scales as for Fig. 1–19.

In female, exopods of P1 to P4 3-segmented; endopods of P1 and P2 1-segmented, of P3 2-segmented, and of P4 3-segmented. A distal pointed process on lateral margin of endopod in S1 of P3 and S2 of P4. Male P1 to P4 similar to those of female, except P3 endopod with three segments and with distal pointed process on lateral margin of S2. Male P5 exopod more elongate than that of previous stage and provided with five spines: two lateral, two medial, and one distal.

Occurrence: January, April, and June–September.



FIGURES 43–53. *Chiridius gracilis.*
Copepodite IV, female: FIG. 43. dorsal view; FIG. 44. lateral view; FIG. 45. antennules; FIG. 46. mandible; FIG. 47. maxillule; FIG. 48. maxilla; FIG. 49. maxilliped; FIG. 50–53. P1–P4. Scales as for Fig. 1–19.

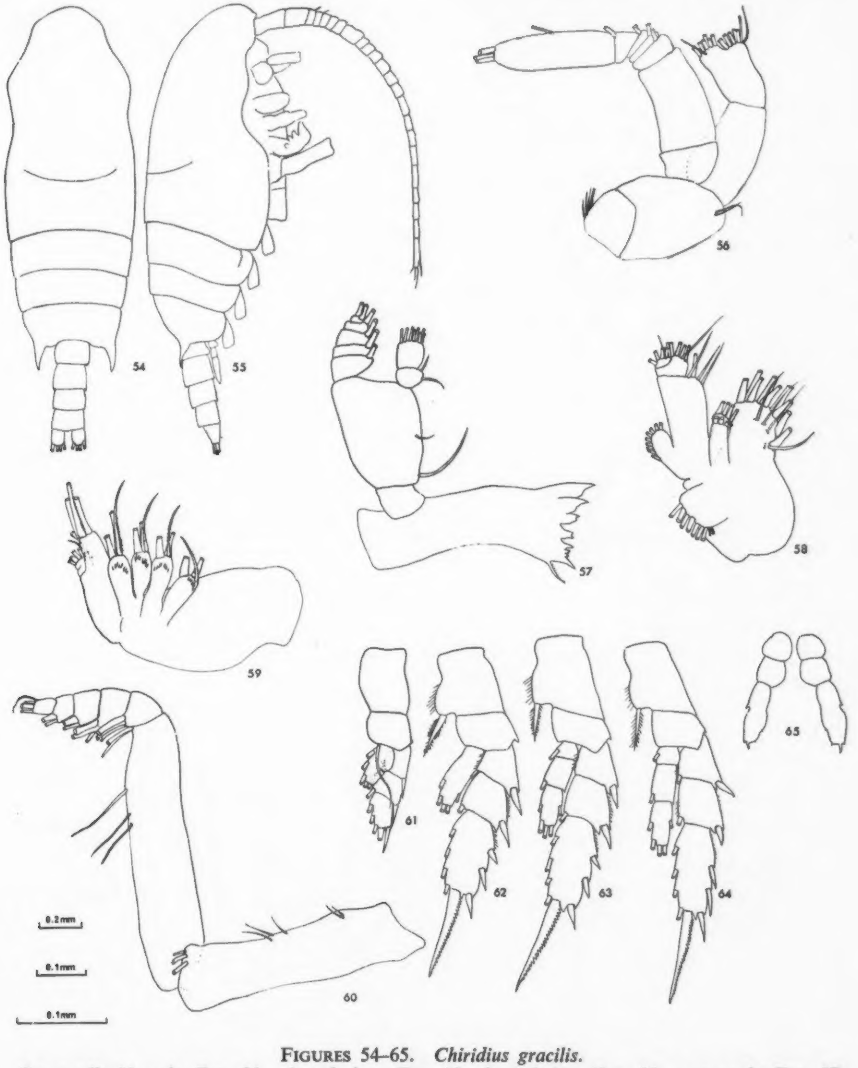
Adult Male

(FIG. 77–90)

Total length 2.45 ± 0.12 mm; prosome 1.82 ± 0.06 mm. Prosome to urosome ratio 3.41 ± 0.11.

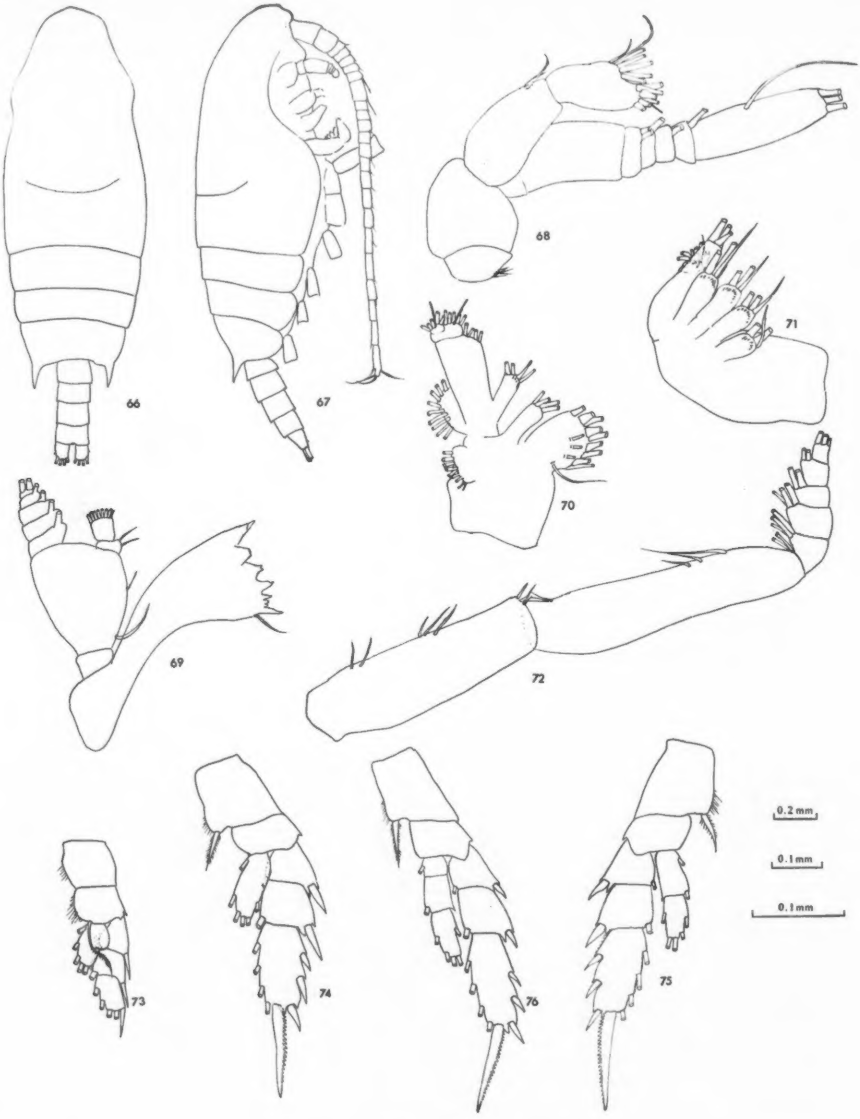
The following description is based on an adult male specimen from the Cabot Strait, IBP Station 3, Sample AF/III(3).

Total length 2.44 mm: cephalothorax, 1.24 mm; metasome, 0.56 mm; urosome, 0.52 mm; caudal rami, 0.12 mm. Greatest width at prosome, 0.72 mm. Anterior profile of prosome narrow, 0.32 mm in width, and nearly truncate. From dorsal view, two lateral body surfaces diverging greatly over antennules. Straight dorsal profile rounded off anteriorly above orifice. Suture between cephalosome and first thoracic



FIGURES 54–65. *Chiridius gracilis.*
Copepodite V, male: FIG. 54. dorsal view; FIG. 55. lateral view; FIG. 56. antennule; FIG. 57. mandible; FIG. 58. maxillule; FIG. 59. maxilla; FIG. 60. maxilliped; FIG. 61–65, P1–P5. Upper scales for whole specimens, middle for legs, and lower for mouthparts.

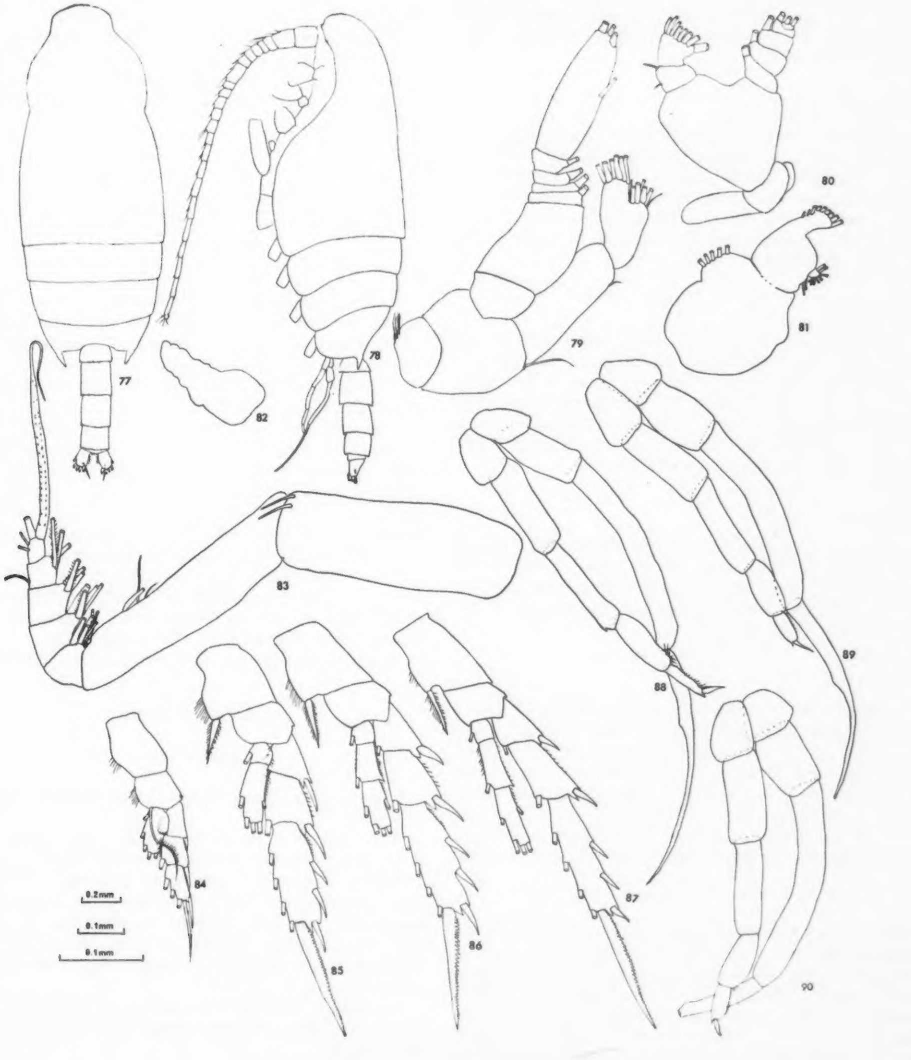
somite indistinct. Metasomal processes slightly bent inwards and reaching posterior margin of Ul. Urosome slender and straight, composed of five free somites with percentages of length 16:32:26:20:6. Ratio of prosome to urosome 3:46. Caudal rami about 1/3 longer than wide.



FIGURES 66–76. *Chiridius gracilis.*
Copepodite V, female: FIG. 66. dorsal view; FIG. 67. lateral view; FIG. 68. antennule; FIG. 69. mandible; FIG. 70. maxillule; FIG. 71. maxilla; FIG. 72. maxilliped; FIG. 73–76, P1–P4. Scales as for Fig. 54–65.

Antennule symmetrical, 23-jointed with fusions of 8th–9th and 24th–25th segments, and reaching to tip of metasomal process. Percentages of lengths of the segments in total length of antennule as follows:

Segment	1	2	3	4	5	6	7	8–9
%	5.6	5.0	2.2	2.4	2.7	2.3	2.6	6.1
Segment	10	11	12	13	14	15	16	17
%	2.7	3.0	3.2	3.6	3.9	4.2	4.9	5.0
Segment	18	19	20	21	22	23	24–25	
%	5.2	6.4	6.5	4.4	6.0	5.5	6.6	



FIGURES 77–90. *Chiridius gracilis.*
Adult male: FIG. 77. dorsal view; FIG. 78. lateral view; FIG. 79. antennule; FIG. 80. mandible; FIG. 81. maxillule; FIG. 82. maxilla; FIG. 83. maxilliped; FIG. 84–88. P1–P5; FIG. 89. P5 of South Pacific specimen; FIG. 90. P5 of Tasman Sea specimen. Scales as for Fig. 54–65.

ANTENNA

Protopod 2-segmented, with weak spinules at medial base of B1. Endopod 2-segmented, slightly more than half as long as exopod; lateral lobe of S2 with six long setae, medial lobe with five long and two short setae. Exopod 7-segmented; S2 3 times S1 and subequal to S7 in length; S3 to S6 ring-like, each segment with a long seta; S7 with three apical setae and a seta near center of segment.

MANDIBLE

Protopod 2-segmented. Endopod 2-segmented; S1 with two setae on medial margin; S2 with eight long and one short medial setae. Exopod 5-segmented, slightly longer than endopod; each of first four segments with a medial seta; S5 with two apical setae. Mandibular gnathobase degenerated to a weak and toothless remnant.

MAXILLULE

Degeneration obvious in size, segmentation, and armature. All inner lobes degenerated. Outer lobe visible but very much reduced and with five setae. Exopod fairly well developed, with seven long curved setae and two small straight setae. Endopod reduced to one small bulb-like segment with eight setae. Protopod S2 with two setae on medial margin.

MAXILLA

Degree of degeneration in size, segmentation, and armature exceeding that of all other cephalic appendages in adult male. Entire appendages reduced to a small blade-like structure bearing several lobular processes without setae.

MAXILLIPED

Protopod B1 rectangular with two short setae distally; B2 about 1/3 longer than B1, with three setae, one long and two short, on medial margin in distal third, and two long distal setae. Setation on 5-segmented endopod 4, 4, 3, 3–1, and 3–1, respectively. One apical seta of distal segment with spinules.

Numbers of segments in P1 to P4 same as those of copepodite V. All segmentations complete and clear.

P1

Protopod B1 without medial setae; B2 with strong S-curved seta near insertion of endopod, and a small, weak spine near distal end of lateral margin. Endopod with lateral tubercle bearing spinules on inferior margin, and with three medial and two terminal setae. Exopod with one lateral spine in S1, one lateral spine and one medial seta in S2, one lateral spine, one smooth terminal spine, and three medial setae in S3.

P2

Protopod B1 with expanded internal margin bearing a seta; B2 with a short spine at lateral margin near insertion of exopod. Endopod with one medial seta in S1, two lateral setae, two medial setae, and one medial seta in S2. Exopod with one lateral spine and one medial seta in S1 and S2, three lateral spines, one serrate terminal spine, and four medial setae in S3.

P3

Protopod similar to that of P2. Endopod with one medial seta in S1 and S2, two medial setae, two terminal setae and one lateral seta in S3; S2 with a pointed process at distal lateral margin. Exopod similar to that of P2.

P4

Whole appendage greatly resembling P3, but slightly narrower and longer.

P5

Asymmetrical and uniramous. Left leg straight, 5-segmented and slightly longer than first three segments of right leg combined; first and second segments naked; third segment with short tooth-like process at lateral distal end; fourth segment with hair-like setae distally on medial margin; fifth segment with hairs on medial margin, and a distal lobular process nearly half as long as segment. Right leg 4-segmented; first and second segments naked, shorter than those of left leg; third segment bent slightly near middle, and almost twice as long as first two segments combined; fourth segment naked, with a small expansion in the proximal third and a thin blade-like distal portion.

P5 of specimens from South Pacific off Chile (Fig. 89) and Tasman Sea (Fig. 90) differ from the above description in lacking lateral distal process in S3 of left leg and in relative lengths of segments.

Occurrence: January, and June to September.

Adult Female

(FIG. 91–101)

General appearance similar to that of adult male and copepodite V.

Total length 2.89 ± 0.21 mm; prosome 2.17 ± 0.11 mm. Anterior profile of cephalothorax bluntly pointed. Metasomal processes directed backwards and reaching end of genital segment. Urosome 4-segmented. Genital segment symmetrical in dorsal view, with a slight swelling on each side at middle of segment, width subequal to length. Ratio of prosome to urosome 3.53 ± 0.14.

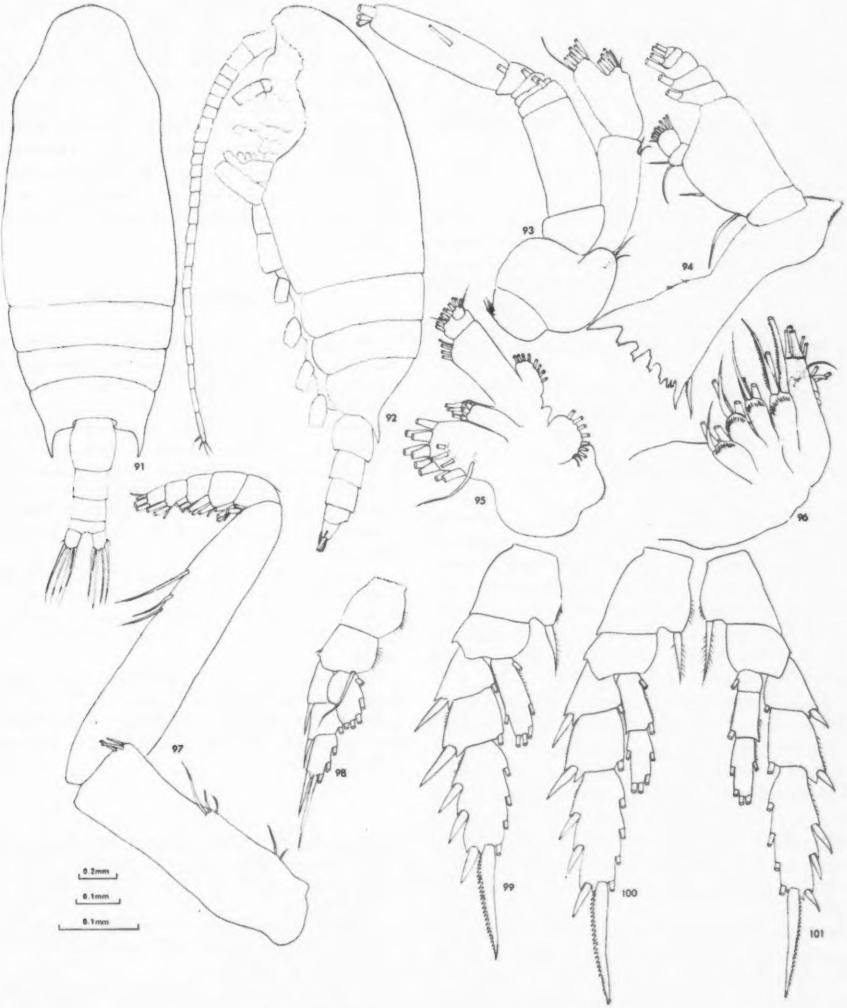
Antennule and antenna similar to those of male. Remaining cephalic appendages showing great sexual dimorphism.

MANDIBLE

Gnathobase strongly developed and nearly twice as long as 2-segmented protopod. Cutting edge with eight teeth. Five anterior teeth strong and, excepting first, bicuspidate; following two weak and bifid; posterior one strong and acuminate. Posterior margin of gnathobase with a spinulose seta near posterior tooth. Protopod B1 small and lacking setae or spines; B2 with a strong arched spine near distal medial margin and a small seta at middle of medial margin. Endopod 2-segmented; S1 with two medial setae, S2 with nine apical setae. Exopod 5-segmented, about twice as long as endopod, with one medial seta on each of first four segments and two apical setae on distal segment.

MAXILLULE

First outer lobe with seven long and two short marginal setae. No second outer lobe. Protopod B2



FIGURES 91–101. *Chiridius gracilis.*
Adult female: FIG. 91. dorsal view; FIG. 92. lateral view; FIG. 93. antennule; FIG. 94. mandible; FIG. 95. maxillule; FIG. 96. maxilla; FIG. 97. maxilliped; FIG. 98–101. P1–P4. Scale as for Fig. 54–65.

with four setae on medial distal end. Exopod short, with eight setae. Endopod 2-segmented, each segment with five strong and one feeble seta. Three inner lobes: first lobe with 13 spines, nine strong and seliform at or near margin, four small on inferior surface and armed with comb-like spinules near their bases; second with four apical setae; third with four apical setae and a few spinules on medial distal margin.

MAXILLA

Each of first to fifth lobes with three setae, one short and slightly curved and two strong and straight. Anterior superior surface of first to fourth lobes with comb-like spinules. Endopod 3-segmented, with 2, 2, and 3 setae on first, second, and third segments, respectively.

MAXILLIPED

Protopod B1 with three lobes, with 2, 3, 3 setae, respectively; B2 with three setae near middle of medial margin and two distal setae. Endopod 5-segmented; S1 and S2 each with three strong and one small seta; S3 and S4 each with two strong and one small seta; S5 with three apical setae and one small lateral seta.

P1 to P4 similar to those of previous stage. P5 wanting.

Occurrence: January, April, and June–September; ovigerous females in June to August.

Discussion

Apart from the difference in size, *C. gracilis* differs markedly from *C. poppei* Giesb. in copepodite V, the only stage of *C. poppei* described (Mazza 1965). In *C. gracilis*, P5 in the male is nearly symmetrical and P3 endopod in the female is 2-segmented; in *C. poppei*, P5 in the male is asymmetrical, with the left leg longer than the right, and P3 endopod in the female is 3-segmented.

Our study shows that morphological changes in *Chiridius gracilis* closely resemble those of the aetideid *Chiridius armatus* (= *Aetideopsis armatus*) as described by Matthews (1964). The major differences between these two species are the presence of a rostrum, separated fourth and fifth metasomal segments and a biramous male P5 in *A. armatus* and absence of a rostrum, fused fourth and fifth metasomal segments, and a uniramous male P5 in *C. gracilis*.

In the adult male of *Chiridius gracilis*, the mandibular gnathobase has degenerated to form a weak toothless structure, the maxillule has changed in size and segmentation, and the maxilla has become reduced to a minute blade-like lobe. The degeneration of adult male mouthparts seems commom among members of the Aetideidae. However, the degree of degeneration of these appendages in the male *C. gracilis* far exceeds that noted by Mazza (1966) in *Gaetanus kruppi* and *Euchirella messinensis*, the only other aetideid species in which the male mouthparts have been described.

The presence and shape of metasomal processes are distinct characters of many species in the family Aetideidae. In *Chiridius gracilis*, as well as in *Gaetanus kruppi* reported by Mazza (1965) and *Aetideopsis armatus* and *Bradyidius bradyi* reported by Matthews (1964), these processes have become fully recognizable in copepodite IV.

The pattern of egg laying exhibits a striking difference between the present study and that of Matthews (1964). The latter reported that the females of the four aetideid species in his investigation shed their eggs directly into the water as does *Calanus finmarchicus*. In our study we found that the ovigerous females of *Chiridius gracilis* retain the eggs on the genital segment in a medially placed ovisac.

Acknowledgments

We thank D. M. Steven (McGill University) and R. J. Conover (Bedford Institute) who made available the collections from the Gulf of St. Lawrence used in this study (IBP Gulf of St. Lawrence project and *Baffin* cruise of May 1968, respectively). For loan of specimens collected elsewhere, we are indebted to J. M. Bradford (New Zealand Oceanographic Institute), J. Fulton (Fisheries and Marine Service, Pacific Biological Station), G. D. Grice (Woods Hole Oceanographic Institution), and M. Omori (University of Tokyo).

We thank Dr E. H. Grainger of the Arctic Biological Station, Fisheries and Marine Service and Dr T. S. Park of the Department of Marine Sciences, Texas A & M University, for critically reviewing the manuscript.

The study was partly supported by a grant to D. C. M. from the National Museum of Natural Sciences, Ottawa. A grant from the Faculty of Graduate Studies and Research, McGill University, is also gratefully acknowledged.

FARRAN, G. P. 1905. Report on the Copepoda of the Atlantic slope off counties Mayo and Galway. Sci. Invest. Fish. Br. Ire., 1902–3, Pt. 2, Append. (2): 23–52.

1908. Second report on the Copepoda of the Irish Atlantic slope. Sci. Invest. Fish. Br. Ire., 1906, Pt. 2. 104 p.

1929. Crustacea. Part X. Copepoda. Nat. Hist. Rep. Terra Nova Exped. (Zool.) 8: 203–306.

FLEMINGER, A. 1967. Taxonomy, distribution and polymorphism in the *Labidocera jollae* group with remarks on evolution within the group (Copepoda: Calanoida). Proc. U.S. Natl. Mus. 120(3567): 1–61.

GRICE, G. D. 1963. Deep water copepods from the western North Atlantic with notes on five species. Bull. Mar. Sci. Gulf Caribb. 13(4): 493–501.

HUMES, A. G., AND R. U. GOODING. 1964. A method for studying the external anatomy of copepods. Crustaceana (Leiden) 6: 238–240.

MATTHEWS, J. B. L. 1964. Bottom living copepods from western Norway. Sarsia 16(1): 1–46.

MAZZA, J. 1965. Le développement de quelques copépodes en Mediterranée II. Les stades de *Gaetanus kruppi* Giesb., *Euchirella messinensis* Cl., *Chiridius poppei* Giesb., *Pseudaetideus armatus* (Boeck) et *Heterorhabdus spinifrons* Cl. Rev. Trav. Inst. Pêches Marit. 29: 283–320.

1966. Evolution de l'appareil buccal au cours du développement post-larvaire des Aetideidae et des Euchaetidae (Copépodes pélagiques) ses incidences sur le sex-ratio des adultes. Vie et Milieu, Série A: Biologie marine 17: 1027–1044.

PARK, T. S. 1970. Calanoid copepods from the Caribbean Sea and Gulf of Mexico. 2. New species and new records from plankton samples. Bull. Mar. Sci. 20: 472–546.

TANAKA, O. 1937. Copepods from the deep water of Suruga Bay. Jap. J. Zool. 7: 251–271.

1957. The pelagic copepods of the Izu region, middle Japan. Systematic Account III. Family Aetideidae (Pt. 1). Publ. Seto Mar. Biol. Lab. 6(1): 31–68.

TANAKA, O., AND M. OMORI. 1970. Additional report on calanoid copepods from the Izu Region. Pt. 3A. *Euaetideus*, *Aetideopsis Chiridius*, *Gaidius* and *Gaetanus*. Publ. Seto Mar. Biol. Lab. 18(2): 109–141.

VERVOORT, W. 1957. Copepods from Antarctic and sub-Antarctic plankton samples. Rep. B.A.N.Z. Antarct. Res. Exped. (B)3: 1–160.

Genetic Differences in Retention of Swimbladder Gas Between Two Populations of Lake Trout (*Salvelinus namaycush*)[1]

P. IHSSEN

Ontario Ministry of Natural Resources
Fish and Wildlife Research Branch, Maple, Ont.

AND J. S. TAIT

Department of Biology
York University, Downsview, Ont. M3J 1P3

IHSSEN, P., AND J. S. TAIT. 1974. Genetic differences in retention of swimbladder gas between two populations of lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 31: 1351–1354.

Lake trout (*Salvelinus namaycush*) derived from two populations differed in retention of swimbladder gas. The two reciprocal interpopulation crosses were intermediate to the parent populations. The two F_1 hybrids obtained by crossing fish from each lake trout population with brook trout (*S. fontinalis*) were also found different in retention. Gas retention was correlated with depth distribution of the parent populations, high retention being associated with deeper distribution.

IHSSEN, P., AND J. S. TAIT. 1974. Genetic differences in retention of swimbladder gas between two populations of lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 31: 1351–1354.

Les touladis (*Salvelinus namaycush*) provenant de deux populations diffèrent sous le rapport de la rétention du gaz de la vessie gazeuse. Les individus des deux croisements réciproques entre les populations sont intermédiaires entre les populations parents. Les deux hybrides de la F_1 obtenus en croisant des poissons de chaque population de touladis avec l'omble de fontaine (*S. fontinalis*) diffèrent également dans la rétention. Nous établissons une relation entre la rétention du gaz et la répartition bathymétrique des populations parents, une haute rétention étant associée à une répartition en plus grande profondeur.

Received March 8, 1974
Accepted May 9, 1974

Reçu le 8 mars, 1974
Accepté le 9 mai, 1974

THE objective of this study was to determine whether genetic differences in the ability to retain swimbladder gas could be demonstrated for lake trout from two different lakes. In salmonid fish, ability to retain gas in the swimbladder has been shown to be correlated with ability to live in deep water. Tait (1959) found that for seven species of salmonids, retained under pressure and prevented from swallowing air, relative ability to keep the swimbladder filled was correlated with depth distribution in their normal habitat. In that study, the lake trout was found to have the best retention and the brown trout (*Salmo trutta*) the poorest. In a field study, Straight (1969) found that groups of splake hybrids (lake trout × brook trout), whose ability to retain swimbladder gas had been previously determined, had depth distributions correlated with their ability to retain gas.

Materials and Methods

Gametes were collected from the lake trout populations of Lake Simcoe and Lake Louisa. Lake Simcoe is a large lake (725 km²) in southern Ontario. Lake Louisa, a smaller lake (6.0 km²), lies 135 km to the northeast in Algonquin Park on the Precambrian Shield. The following crosses were produced by artificial fertilization:

$L^L \times L^L$
$L^S \times L^S$
$B \times L^S$
$L^L \times L^S$
$L^S \times L^L$
$B \times L^L$

For each cross the female parent is written first and L and B stand for lake and brook trout, respectively.

---

[1] Contribution No. 74–7 of the Ontario Ministry of Natural Resources, Fish and Wildlife Research Branch, Maple, Ont.

Printed in Canada (J3346)
Imprimé au Canada (J3346)

Superscripts on the Ls refer to Louisa and Simcoe, the lakes of origin of the lake trout parents. Brook trout eggs were taken from a domestic strain held in our laboratory.

The fish were reared under identical hatchery conditions to age 23 mo (mean total length for lake trout 205 mm, for F_1s 204 mm) when testing of their ability to retain swimbladder gas was begun. The method of measurement of this character was the one described by Tait (1970). Briefly, the technique for each test involves subjecting the fish to a pressure of 1.4 atm for 12 days without access to an air surface. Then the fish are anesthetized and their buoyancy is measured by slowly reducing the pressure. The units of measurement are cm of Hg which represent the pressure at which the fish are just buoyant, the flotation pressure. From each family of the F_1 crosses, a sample of about 20 fish was tested. There were four families of the type B × L^L and three of the type B × L^S. From each of the four types of lake trout crosses, a sample of about 15 fish was tested. These samples of lake trout came from lots that were derived from several males and females. The lake trout crosses were tested three times and the F_1 families twice.

Analyses of variance were performed to test for differences in flotation pressure associated with the origin of the two parent stocks of lake trout.

Results

In each test the Lake Simcoe lake trout ($L^S \times L^S$) had higher flotation pressures than the Lake Louisa lake trout ($L^L \times L^L$); the interpopulation lake trout crosses were intermediate (Table 1). All the F_1 families derived from the L^S males had higher flotation pressures than the families derived from L^L males (Table 2).

The analysis of variance on the lake trout data gave a male effect significant at the 1% level ($P < 0.01$) and a female effect significant only at about the 7% level ($0.05 < P < 0.10$) with the interaction between males and females non-significant ($0.10 < P < 0.25$). The components of variance expressed as percentages of the total phenotypic variance were 3% and 24% for the female and male effect, respectively. The replicate experiments performed over a period of about 6 mo showed a progressive increase in flotation (Table 1). The differences among tests were highly significant ($P < 0.01$) implying that during the 6 mo spanned by tests 1–3 (Table 1) the flotation pressure increased significantly. The same effect was noted for the F_1s (Table 2) tested 4 mo apart.

For the F_1 hybrids, as for the lake trout, a highly significant effect associated with the origin of the lake trout male parent was found. The F_1 hybrids of Lake Simcoe paternal origin (B × L^S) retained gas better (i.e. had higher flotation pressures) than the F_1 hybrids of Lake Lousia paternal origin (B × L^L) (Table 2). The component of variance associated with the male parent was 25%. Comparing the magnitude of the male effect in the lake trout crosses in Table 3 (2.6 cm of Hg on L^S ♀s, 1.4 cm of Hg on L^L ♀s) with that in F_1 crosses (2.9 cm of Hg), the male effect appears to be larger for the F_1 hybrids.

For the F_1 crosses, since individual families were tested, the variance components for within and among families (full sibs) within the two types of F_1s (B × L^L, B × L^S, Table 2) could be estimated from the ANOVA. The intraclass correlation, calculated from the above variance components gave a value of the heritability, h^2, for this character within the two populations, of $h^2 = 0.58 \pm 0.21$[2]. This estimate of h^2 includes half of the dominance variance and all of the maternal effect (Kempthorne 1957) and hence is probably somewhat inflated.

TABLE 1. Mean flotation pressures with their standard errors for the lake trout crosses in centimeters of Hg.

Female parent	Test 1 Nov. 1972		Test 2 Jan. 1973		Test 3 Apr. 1973	
	Male parent					
	L^S[a]	L^L[b]	L^S	L^L	L^S	L^L
L^S	42.00±0.28 210[c]	39.94±0.55 216	42.69±0.50 217	40.39±0.80 221	45.00±0.43 225	41.57±0.57 225
L^L	41.40±0.37 200	39.89±0.78 192	40.90±0.31 211	40.00±1.14 200	43.40±0.45 218	42.20±0.24 208

[a] Parent from the Lake Simcoe population.
[b] Parent from the Lake Louisa population.
[c] Mean total length in mm.

TABLE 2. Mean flotation pressures with their standard errors for the F_1 hybrids in centimeters of Hg.

B×L^S[a]			B×L^L[b]		
Family[c]	Test 1 Sept. 1972	Test 2 Feb. 1973	Family	Test 1 Sept. 1972	Test 2 Feb. 1973
1	37.60±0.33 212[d]	37.30±0.77 244	4	33.63±0.72 198	35.63±0.46 229
2	37.20±0.54 211	38.26±0.65 239	5	35.21±0.40 208	36.26±0.44 236
3	37.00±0.43 201	38.83±0.94 237	6	33.74±0.83 204	35.00±0.85 231
			7	35.94±0.79 197	36.63±1.05 219

[a] Crosses obtained from brook trout females × Lake Simcoe lake trout males.
[b] Crosses obtained from brook trout females × Lake Louisa lake trout males.
[c] Each family is one set of full sibs.
[d] Mean total length in mm.

The value of h^2 for the same character in F_2 splake hybrids given by Butler (1968) without the dominance or maternal effect is 0.20.

Discussion

The difference in the ability to retain swimbladder gas between Lake Simcoe and Lake Louisa lake trout as measured by their buoyancy after exposure to hydrostatic pressure is 2.8 cm Hg (Table 3). The total range for this character found within the genus *Salvelinus* is about 23 cm Hg represented by the difference between brook trout and lake trout (Tait 1970). Hence the intraspecific stock difference is quite substantial, representing about 12% of the intrageneric range for this character. Since this difference was found among fish stocks reared and maintained under identical environmental conditions and also since this effect was seen in interpopulation as well as interspecific hybrids, it must be concluded that it is genetic.

Since flotation pressure has been shown to be correlated with depth distribution, we compared the distribution of lake trout in lakes Simcoe and Louisa. The two lakes are very different in their physical limnology. The thermocline is much deeper in Lake Simcoe than in Lake Louisa. The 10 C isotherms during July are at about 8 and 20 m for lakes Louisa and Simcoe, respectively. During the summer, the lake trout of Lake Simcoe are distributed below the thermocline 25–35 m deep or more, and, during the winter, they are found primarily at 15–20 m (MacCrimmon and Skobe 1970). In Lake Louisa, Martin (1951) found that, during the summer months of thermal stratification, the depth distribution of lake trout is widespread with a concentration between the 8 and 10 C isotherms at about 10–20 m deep. Fish were also found in much shallower water up to the 18 C isotherms in the 5–10 m depth zone. The deeper distribution is in the lake with the deeper thermocline. Although lake trout are generally considered to be deep-swimming, they do not necessarily go deep in lakes that have shallow thermoclines or undergo little surface warming. There are several reports of lake trout occurring near the surface in mid-summer in northern lakes such as Great Bear Lake (Miller and Kennedy 1947) and Lake Vojmsjön, Sweden (Nilsson and Särdson 1968). Although no detailed information on the winter depth distribution in Lake

TABLE 3. Mean flotation pressures with their standard errors over the three tests for the lake trout and over the two tests and families for the F_1 hybrids.

Female parent	Male parent	
	L^S	L^L
I_S[a]	43.23±0.25(38)[d]	40.63±0.37(37)
I_L[b]	41.90±0.22(60)	40.70±0.47(45)
B[c]	37.70±0.36(103)	34.76±0.42(147)

[a] Lake Simcoe lake trout.
[b] Lake Louisa lake trout.
[c] Domestic brook trout.
[d] The number of observations on which the mean is based.

---

[2] In the calculation of the standard error, normality of the intraclass correlation is assumed.

Louisa is available, it is believed that during the winter the depth distribution is probably even shallower such as it is in Redrock Lake, another Precambrian Shield lake of similar size in the same geographic area (Martin 1951). Hence the flotation values for these two populations are correlated to their depth distribution so that the population that must live in deeper water if it is to avoid warm summer temperatures has the greater ability to retain swimbladder gas. It appears that adaptation to the two differing lake environments has led to the observed genetic difference.

Genetic differences among lake trout populations usually have been inferred from differences in morphological characters for adult specimens. For example, Khan and Quadri (1971) found that comparisons of morphometric characters of lake trout from various North American drainages suggest the development of different gene pools. Since morphological characters are greatly influenced by the environment, differences in these characters may not always reflect genetic differences.

The demonstration that different populations of the same species represent different physiological races may have important management applications. The implications of this concept to management practices for Pacific salmon have been evaluated recently (Simon and Larkin 1972). Valuable distinct genetic stocks may be lost by not recognizing that a species, even within a relatively small geographic area, can be represented by several specially adapted stocks.

Acknowledgments

We thank J. E. Dewar for technical assistance and F. E. J. Fry and A. H. Lawrie for critical reading of the manuscript.

BUTLER, L. 1968. The potential use of selective breeding in the face of changing environment, p. 54–72. *In* K. H. Loftus [ed.] A Symposium on Introduction of Exotic Species. Ont. Dep. Lands Forests Res. Rep. No. 82.

KEMPTHORNE, O. 1957. An introduction to genetic statistics. John Wiley and Sons Inc., New York, N.Y. 468 p.

KHAN, N. Y., AND S. U. QADRI. 1971. Intraspecific variations and postglacial distribution of lake char (*Salvelinus namaycush*). J. Fish. Res. Board Can. 28: 465–476.

MACCRIMMON, H. R., AND E. SKOBE. 1970. The fisheries of Lake Simcoe. Ont. Dep. Lands Forests. 140 p.

MARTIN, N. V. 1951. A study of the lake trout, *Salvelinus namaycush*, in two Algonquin Park lakes. Trans. Am. Fish. Soc. 81: 111–137.

MILLER, R. B., AND W. A. KENNEDY. 1947. Observations on the lake trout of Great Bear Lake. J. Fish. Res. Board Can. 7: 176–189.

NILSSON, N.-A., AND G. SVÄRDSON. 1968. Some results of the introduction of lake trout (*Salvelinus namaycush* Walbaum) into Swedish lakes. Inst. Freshw. Res. Drottningholm Rep. No. 48: 5–16.

SIMON, R. C., AND P. A. LARKIN. 1972. The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries. The University of British Columbia, Vancouver, B.C. 000 p.

STRAIGHT, W. J. 1969. Depth distribution of splake of known ability to retain swimbladder gas. M.Sc. Thesis. York Univ., Downsview, Ont. 45 p.

TAIT, J. S. 1959. Swimbladder studies in salmonoids relating to problems of depth. Ph.D. Thesis. Univ. Toronto, Toronto, Ont. 52 p.

1970. A method of selecting trout hybrids (*Salvelinus fontinalis* × *S. namaycush*) for ability to retain swimbladder gas. J. Fish. Res. Board Can. 27: 39–45.

Taxonomic Status of the *Salvelinus alpinus* Complex

S. U. Qadri

Department of Biology
University of Ottawa, Ottawa, Ont.

Qadri, S. U. 1974. Taxonomic status of the *Salvelinus alpinus* complex. J. Fish. Res. Board Can. 31: 1355–1361.

Minor differences in osteology and morphology were observed in the eastern North American species of charrs *Salvelinus aureolus* (Sunapee), *S. marstoni* (Quebec red), and *S. oquassa* (blueback). The coefficient of difference values, which determine the degree of overlap were lower than 1.28 for the total numbers of vertebrae, ribs, epineurals, gill rakers, branchiostegals, pores on the lateral line scales, and also for many morphological characters suggesting that the three chars are conspecific and should by synonymized as *S. alpinus oquassa.*

Qadri, S. U. 1974. Taxonomic status of the *Salvelinus alpinus* complex. J. Fish. Res. Board Can. 31: 1355–1361.

L'auteur a observé des différences mineures dans l'ostéologie et la morphologie des espèces de truites de l'est de l'Amérique du Nord *Salvelinus aureolus* (Sunapee), *S. marstoni* (rouge du Québec) et *S. oquassa* (dos bleu). Les coefficients de différence, déterminant le degré de chevauchement, ont des valeurs inférieures à 1.28 pour le nombre total de vertèbres, côtes, épineuraux, branchicténies, rayons branchiostèges, pores sur les écailles de la ligne latérale, ainsi que pour plusieurs caractères morphologiques, ce qui donne à croire que les trois truites sont conspécifiques et devraient être synonymisées sous le nom de *S. alpinus oquassa.*

Received March 10, 1971
Accepted April 29, 1974

Reçu le 10 mars, 1971
Accepté le 29 avril, 1974

The present detailed osteological and morphological study was planned to clarify the status of the Quebec red (*Salvelinus marstoni*), blueback (*S. oquassa*), and Sunapee (*S. aureolus*) and also to establish their interrelationships.

In eastern North America, isolated populations of the Quebec red occur in the province of Quebec, the blueback in Maine, and the Sunapee in Maine and New Hampshire (Waters 1960)

From the earliest times, ichthyologists have recognized four species in the genus *Salvelinus:* the Arctic char (*S. alpinus* or its complex), the brook char (*S. fontinalis*), the Dolly Varden (*S. malma*), and the lake char (*S.(c). namaycush*). Henn and Rinkenbach (1925) included the aurora char (*S. timagamiensis*) in the genus; Qadri (1968) reduced it to a subspecies. The blueback and the Sunapee were considered distinct species by Kendall (1914) and Waters (1960). Vladykov (1954) suggested that *S. aureolus* and *S. marstoni* are distinct species and that the latter is conspecific with *S. oquassa*. Vladykov (1957) divided *S. marstoni* into three subspecies, *S. m. marstoni*, *S. m. intermedius*, and *S. m. cavanaghi*. Dymond (1947) and Legendre (1954) considered *S. marstoni* a subspecies of *S. alpinus.*

Printed in Canada (J2075)
Imprimé au Canada (J2075)

Methods and Materials

Number, size, and source of the fish studied are shown in Table 1. For osteological studies specimens were cleared in 1–2% KOH and stained with alizarin red S following the method of Evans (1948). All morphometric measurements and meristic counts were made on the left side of the fish.

The positions and shapes of all cartilages and chondral and dermal bones of the chondrocranium were studied. Only the following important bones, which showed promise in salmonid taxonomy (Qadri 1964), were considered: supraethmoid, frontal, premaxilla, maxilla, supramaxilla, dentary, vomer, lingual plate, and caudal skeleton.

The alveoles, representing missing teeth on the premaxilla, maxilla, dentary, vomer, and lingual plate were included in the count with the teeth. On the premaxilla and maxilla, up to seven replacement teeth were embedded in the connective tissue lateral to the first series but were not included in the count; on the dentary a maximum of eight such teeth were present.

Figure 1 shows the measurements taken on all the above bones except the caudal skeleton. The bone terminology used here is that used by Norden (1961) and Qadri (1964). Since most measurements on bones overlapped considerably, statistics on them were not taken. All the bones photographed were from adult female fishes.

Twenty morphometric measurements (Ouellette and Qadri 1966) were taken on each fish but only the nine important ones were used for this study; these measurements were adopted from Vladykov (1954) and Hubbs and Lagler (1958): FL — fork length; BdL — body length from anterior upper end of opercle to fork of tail; pDF-distance from insertion of dorsal fin to fork of tail; P–V — distance between origin of pectoral and ventral fins; V–A — distance from origin of ventral fin to origin of anal fin; D–ad — distance from insertion of dorsal fin to origin of adipose fin; H — head length; I — interorbital width; ma–P — distance from anterior tip of lower jaw to origin of pectoral fin. Seven of the above measurements were changed into percentages of the fork length. The eighth, interorbital width, was transformed as a percentage of the head length.

Of the 24 meristic characters (Qadri 1964) counted on each fish, only the following six which showed some differences were considered. The meristic counts were made on stained specimens. Total number of the vertebral centra (urostyle not included), ribs, epineurals, gill rakers on the first left gill arch, branchiostegals, and pores on the lateral line scales were counted.

The data on morphometric and meristic characters were subjected to analysis of variance by using the $R \times 2$ method of classification (Steel and Torrie 1960). By this treatment the effect of groups of fishes, their sex, and their interactions were considered. A 1% level of significance was used. Only the effect of groups, but not the sex, showed a significant difference at 1%. Whenever the F values were found significant, the means for a particular character for various groups were compared with the help of Duncan's (1955) multiple range test as modified by Kramer (1956) for unequal subclasses.

The coefficients of difference (CD =

$$\frac{\text{Mean for population b} - \text{Mean for population a}}{\begin{array}{c}\text{Standard deviation}\\ \text{for population a}\end{array} - \begin{array}{c}\text{Standard deviation for}\\ \text{population b}\end{array}}),$$

for several morphometric and meristic characters of fish were calculated as prescribed by Mary et al. (1953), values of 1.28 and higher being considered indicative of subspecific status. At this value 90% of the fish in each of two populations being compared differed from one another.

Results and Discussion

Morphological studies of the genera *Salvelinus*, *Salmo*, and *Oncorhynchus* were done by Richardson

TABLE 1. Number, size, and source of fish studied.

Locality		Osteology		Morphometry		Meristics	
		No.	fork length *mm*	No.	fork length *mm*	No.	fork length *mm*
			S. marstoni				
York Lake, Que.	48°58′N 65°26′W	–	–	18	170–290 (238.9)	–	–
Harriman Lake, Que.	48°14′N 65°50′W	–	–	10	270–296 (283.4)	–	–
Large Davis Lake, Que.	47°49′N 71°08′W	–	–	10	135–188 (161.5)	10	135–188 (161.5)
Saint Germain Lake, Que.	45°47′N 75°48′W	5	224–253 (233.6)	5	224–253 (233.6)	5	224–253 (233.6)
Battle Lake, Que.	45°40′N 75°35′W	4	291–470 (393.3)	5	291–473 (394.0)	–	–
			S. oquassa				
Pushineer Pond, Maine	46°50′N 68°50′W	21	198–245 (215.0)	25	136–296 (212.5)	23	136–245 (210.0)
			S. aureolus				
Floods Pond, Maine	44°44′N 68°30′W	20	184–282 (242.0)	23	184–302 (244.4)	20	184–282 (241.1)

(1836), Parker (1873), Gregory (1933), Tchernavin (1923, 1937), Vladykov (1954, 1962, 1963), Morton and Miller (1954), McPhail (1961), Norden (1961), and Reshetnikov and Savvaitova (1962). But the problem of minor morphological changes due to environmental effects was not discussed sufficiently

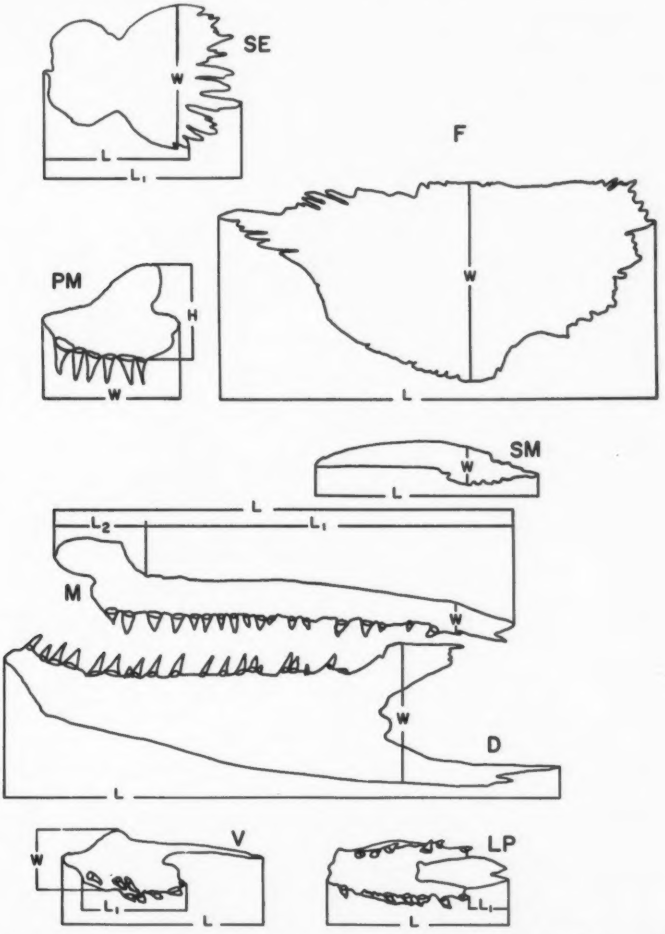


FIG. 1. Measurements on dermal skull bones. Abbreviations — SE, dorsal view of the supraethmoid; L, maximum length at the lateral side, L_1, maximum length, W, maximum width. F, dorsal view of frontal; L, maximum length, W, maximum width. PM, lateral view of the premaxilla; H, maximum height, W, maximum width. M, lateral view of the maxilla; L, maximum length, L_1, length of body of the maxilla, L_2 length of head of the maxilla, W, maximum width at posterior flat part of bone without teeth. SM, lateral view of the supramaxilla; L, maximum length, W, maximum width. D, lateral view of the dentary; L, maximum length, W, maximum width at posterior end. V, lateral view of the vomer; L, maximum length, L_1, combined length of head and crest of bone, W, maximum width of bone in head region. LP, dorsal view of the lingual plate; L, maximum length, L_1, distance between end of tooth row and posterior end of bone.

TABLE 2. Measurements (expressed as percentages) taken on bones as shown in Fig. 1 and total numbers of teeth and sensory pores on bones.

Fish species	Quebec red *S. marstoni*	Blueback *S. oquassa*	Sunapee *S. aureolus*
Supraethmoid			
$\frac{L}{L_1}$	89.2–112.6	70.0–100.0	76.0–89.0
	101.5	85.7	81.3
$\frac{W}{L_1}$	35.6–63.7	36.4–58.8	34.0–51.9
	46.2	47.8	40.4
Frontal			
$\frac{W}{L}$	43.7–50.6	40.0–46.0	35.3–45.5
	47.1	43.1	41.1
sensory pores	6–10	6–9	6–9
	7.5	7.9	7.6
Premaxilla			
$\frac{H}{W}$	53.3–76.4	58.8–81.7	60.8–88.9
	68.1	68.7	72.9
teeth	6–8	6–9	6–9
	6.9	7.1	7.6
Maxilla			
$\frac{L_1}{L}$	85.7–92.6	79.4–89.0	85.4–92.1
	89.1	85.8	88.5
$\frac{L_2}{L}$	15.6–17.9	16.6–22.1	11.8–22.2
	17.1	19.0	18.5
$\frac{W}{L}$	4.8–9.7	6.8–13.2	7.3–13.3
	8.0	10.3	9.2
teeth	18–25	13–24	15–28
	21.7	18.5	16.6
Supramaxilla			
$\frac{W}{L}$	15.7–22.8	19.1–25.0	25.4–30.1
	19.6	20.5	27.3
Dentary			
$\frac{W}{L}$	22.2–28.5	25.4–30.1	25.0–32.3
	26.5	27.3	27.8
teeth	14–22	15–18	13–21
	17.3	16.6	17.5
sensory pores	4–6	5–7	5–7
	4.8	5.7	5.6
Vomer			
$\frac{L_1}{L}$	43.2–60.9	24.8–61.5	43.1–71.4
	49.5	44.7	52.8
$\frac{W}{E}$	26.6–45.5	21.4–40.6	26.9–52.5
	36.2	33.2	35.5
teeth	6–8	5–8	4–8
	7.3	6.1	6.2
Lingual plate			
$\frac{L_1}{L}$	28.2–50.0	25.3–42.5	28.7–44.9
	34.7	32.4	33.9

(*Continued*)

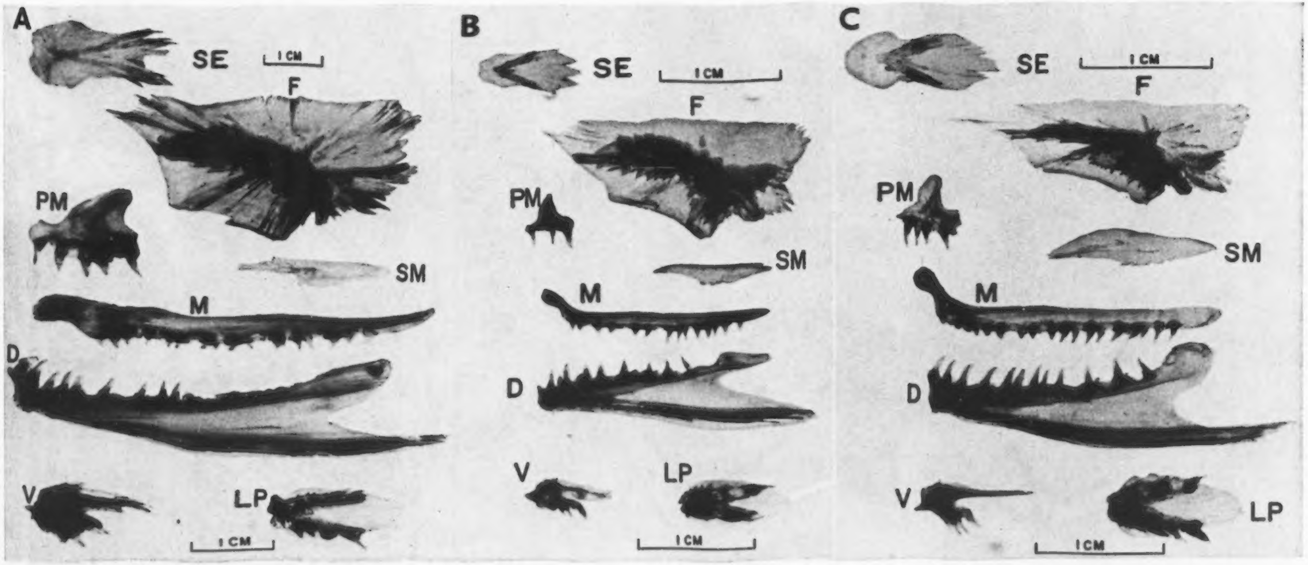

A
SE
1 CM
F
PM
SM
M
D
V
LP
1 CM
B
SE
1 CM
F
PM
SM
M
D
V
LP
1 CM
C
SE
1 CM
F
PM
SM
M
D
V
LP
1 CM

FIG. 2. Dermal skull bones of A, *Salvelinus marstoni* (291 mm FL); B, *S. oquassa* (245 mm FL); C, *S. aureolus* (251 mm FL.) The magnifications for vomer (V) and lingual plate (LP) in A, B, and C are different than those for the remaining bones. See abbreviations in Fig. 1.

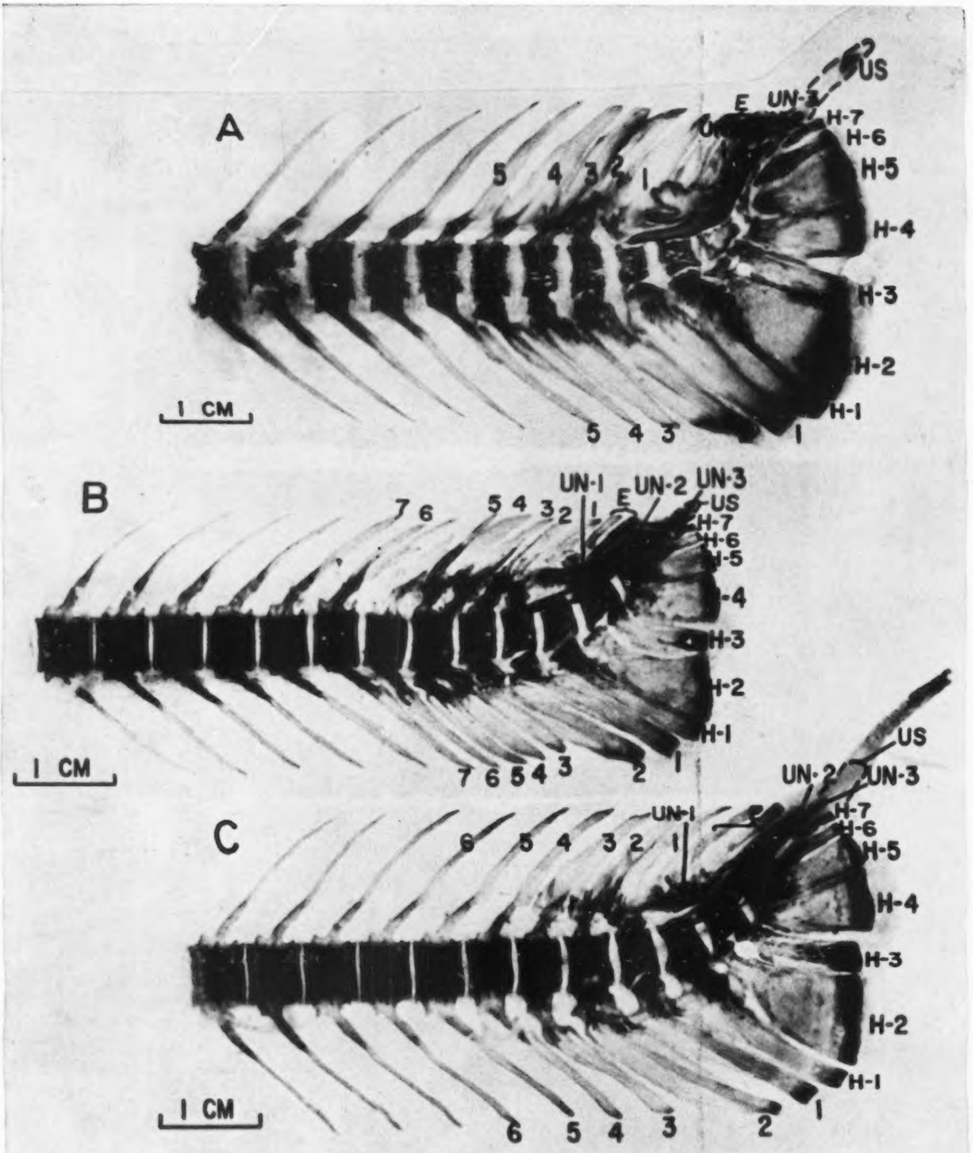

A
B
C
1 CM
E
UN-1
UN-2
UN-3
US
H-1
H-2
H-3
H-4
H-5
H-6
H-7
1
2
3
4
5
6
7

FIG. 3. Caudal skeletons of A, *Salvelinus marstoni*; B, *S. oquassa*; C, *S. aureolus*. E, epural; H, hypural; UN; uroneural; US, urostyle; 1–7 expanded neural (dorsal) and hemal (ventral) spines.

Qadri — J. Fish. Res. Board Can.

TABLE 2. Measurements (expressed as percentages) taken on bones as shown in Fig. 1 and total numbers of teeth and sensory pores on bones. — (*Concluded*)

Fish species	Quebec red *S. marstoni*	Blueback *S. oquassa*	Sunapee *S. aureolus*
teeth	5–10	5–8	5–7
left row	7.1	6.1	6.0
right row	6–8	4–7	5–7
	6.9	5.9	6.0
Caudal skeleton			
max. width of uroneural–1	25.4–38.8	27.3–43.2	24.8–42.9
max. length of uroneural–1	32.1	33.8	31.1
max. length of uroneural–3	56.6–86.4	30.8–88.9	48.3–78.0
max. length of uroneural–2	71.7	63.1	64.4
no. epurals	2–2	1–2	1–2
	2.0	1.9	1.9
no. expanded	6–8	5–9	7–10
neural processes	6.7	6.7	8.0
no. expanded	7–10	5–10	5–11
Hemal processes	8.0	7.4	8.6

by them. Such a study is essential in deciding intraspecific taxa in the genus *Salvelinus*.

Most of the cartilages and chondral bones of the chondrocranium (Fig. 1 and 2 and Table 2) are somewhat similar in shape in the three fishes as suggested by Vladykov (1954); Norden (1961); and Qadri (1964). Of the dermal roofing bones of the skull the supraethmoid and frontal showed minor but overlapping differences between the means of various proportions. For the lateral skull bones (premaxilla, maxilla, supramaxilla, and dentary, etc.) only the average value of $\frac{W}{L}$ of the supramaxilla was maximum in the Sunapee and distinguished it from the other chars In the vomer and lingual plate, used extensively in salmonid taxonomy, the proportions overlapped markedly in the three chars.

In the caudal skeleton (Fig. 3) the values of the proportions $\frac{\text{maximum length of uroneural -3}}{\text{maximum length of uroneural -2}}$ and $\frac{\text{maximum width of uroneural -1}}{\text{maximum length of uroneural -1}}$ and the numbers of expanded neural and hemal processes overlapped considerably in the three chars.

On the basis of the similarity of the dermal skull bones and the caudal skeleton, it was apparent (Table 2) that the Quebec red, blueback, and Sunapee were similar and should be considered conspecific.

Four morphological differences segregated the Sunapee from the Quebec red and blueback (Table 3). However, CD values lower than 1.28 among the three chars (except blueback vs. Sunapee for $\frac{\text{D-ad}}{\text{FL}}$, value 1.36) showed considerable overlap in observations and reduced the importance of these differences, indicating that the chars were similar.

Differences in the relative growth rates of body parts for various forms of *S. alpinus* and *S. malma* were reported by McPhail (1961), but the differences were not great enough to assign scientific names to these forms. Savvaitova (1961) showed that various forms of *S. alpinus* lead different kinds of lives and this is manifested in their morphology. Nikol'skii (1961) mentioned differences in various forms of *S. alpinus* from the Arctic Ocean and its islands and also of *S. salvelinus* from the lakes in the Alps. Barsukov (1960) observed small heads in chars of the eastern Chukotia.

The above-mentioned observations by earlier workers suggested that the morphological differences observed by me in chars were most likely environmental, and that the Quebec red, blueback, and Sunapee belonged to a single species group.

Of the meristic characters only the total numbers of branchiostegals and the scales on the lateral line were helpful in distinguishing the Sunapee from the other chars (Table 3). However, the CD values were all less than 1.28, suggesting that the three chars were conspecific.

The geographic isolation of the Quebec red, blueback, and Sunapee in eastern North America (southern Quebec, Maine, and New Hampshire) from the rest of the Arctic char in N. America suggests another explanation that the three forms be considered a subspecies.

The species previously called *S. marstoni* (Garman 1893) was considered a subspecies (*S. alpinus marstoni*) of the Arctic char by Dymond (1947) and Legendre (1954).

Here, *S. a. marstoni* is renamed as *S. alpinus oquassa* because of precedence of the name *S.*

TABLE 3. Separation of the chars into groups based on morphometric and meristic characters.[a] Whenever the F values were found to be significant at 1%, samples were treated by the Duncan's multiple range test for the means as modified by Kramer. Underlined samples are not significantly different. Range, mean, and one standard error are given for each species.

$\frac{H}{FL}$	%	O 19.6±0.20	M 19.9±0.14	Au 21.3±0.16
$\frac{BdL}{FL}$	%	Au 84.3±0.29	O 85.2±0.28	M 85.3±0.17
$\frac{pDF}{FL}$	%	Au 45.8±0.25	M 47.9±0.23	O 48.3±0.22
$\frac{V\text{–}A}{FL}$	%	Au 19.3±0.24	M 21.0±0.25	O 21.1±0.23
$\frac{D\text{–}ad}{FL}$	%	Au 20.7±0.20	M 21.9±0.19	O 23.8±0.26
$\frac{P\text{–}V}{FL}$	%	M 28.4±0.18	O 30.9±0.32	Au 31.2±0.28
$\frac{ma\text{–}P}{FL}$	%	O 19.4±0.27	M 20.1±0.19	Au 21.4±0.19
$\frac{I}{H}$	%	O 33.0±0.46	M 33.3±0.29	Au 33.4±0.32
No. of vertebrae		Au 63.7±0.27	M 64.1±0.33	O 64.5±0.31
No. of ribs		M 36.2±0.22	O 37.6±0.24	Au 37.8±0.19
No. of epineurals		Au 28.5±0.37	O 29.1±0.32	M 29.2±0.29
No. of gill rakers		Au 20.7±0.15	M 21.3±0.32	O 21.4±0.23
No. of branchiostegals		O 9.6±0.10	M 9.9±0.20	Au 10.6±0.14
No. of lateral line scales with pores		Au 127.1±2.82	M 135.5±0.54	O 136.4±1.80

[a]Letters used for scientific names: Au — *S. aureolus*; O — *S. oquassa*; M — *S. marstoni*.

oquassa (Girard 1854) over *S. marstoni*. The other two forms, previously known as *S. oquassa* and *S. aureolus* (Bean 1887), are synonymized with *S. a. oquassa* from Quebec.

Acknowledgments

Mr J. Cuerrier of the Department of the Environment, Ottawa and Mr C. Paré of the Department of Fish and Game, Quebec, provided the Quebec red. Dr W. H. Everhart, Mr K. Warner, and Mr K. Havey, all of the Department of Inland Fisheries and Game, State of Maine, made possible the collection of the blueback and Sunapee. Mr G. Ben-Tchavtchavadze of this department took the photographs.

Barsukov, V. V. 1960. Contribution to the taxonomy of the Chukotian charrs, genus *Salvelinus*. Problems of Ichthyology, ed. Vopr. Ikhtiol. 14: 3–17.

Bean, T. H. 1887. Description of a supposed new species of charr (*Salvelinus aureolus*) from Sunapee Lake, New Hampshire. Proc. U.S. Natl. Mus. 10: 628–630.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1–42.

Dymond, J. R. 1947. A list of the freshwater fishes of Canada east of the Rocky Mountains. Roy. Ont. Mus. Zool. Misc. Publ. No. 1: 1–36.

Evans, H. E. 1948. Clearing and staining small vertebrates in toto, for demonstrating ossification. Turtox News 26: 42–47.

Garman, S. 1893. The Lac de Marbre trout, a new species. Science 22: 23.

Girard, C. 1854. *Salmo oquassa* Girard. Proc. Boston Soc. Nat. Hist. 4: 262–263.

Gregory, W. K. 1933. Fish skulls: A study of the evolution of natural mechanism. Trans. Am. Philos. Soc. (Phila.) 23(2): 74–481.

Henn, A. W., and W. H. Rinkenbach. 1925. Description of the aurora trout (*Salvelinus timagamiensis*) a new species from Ontario. Ann. Carnegie Mus. 16: 131–141.

Hubbs, C. L., and K. F. Lagler. 1958. Fishes of the Great Lakes. Cranbrook Inst. Sci. Bull. No. 26: 213 p. (revised)

Kendall, W. C. 1914. The fishes of New England. The salmon family: 1. The trout or chars. Mem. Boston Soc. Nat. Hist. 8: 1–103.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal numbers of replications. Biometrics 12: 307–310.

Legendre, V. 1954. Key to game and commercial fishes of the Province of Quebec. The freshwater fishes. Fish. Game Dep. Qué. 1: 1–180.

Mayr, E., E. G. Linsley, and R. L. Usinger. 1953. Methods and principles of systematics zoology. McGraw-Hill Book Co., New York, N.Y. 336 p.

McPhail, J. D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. J. Fish. Res. Board Can. 18: 793–816.

Morton, W. M., and R. R. Miller. 1954. Systematic position of the lake trout, *Salvelinus namaycush*. Copeia 2: 116–124.

Nikol'skii, G. V. 1961. Special ichthyology. Published for the National Foundation Washington, D.C., and the Smithsonian Institution by the Israel Program for Scientific Translation, Jerusalem. 538 p.

Norden, C. R. 1961. Comparative osteology of representative salmonid fishes, with particular reference to the grayling (*Thymallus arcticus*) and its phylogeny. J. Fish. Res. Board Can. 18: 679–791.

Ouellette, R. P., and S. U. Qadri. 1966. Principal component analysis and pattern of growth in *Cristivomer namaycush*. Growth 30: 285–293.

Parker, W. K. 1873. On the structure and development of the skull of salmon. Philos. Trans. R. Soc. London 163: 95–145.

Qadri, S. U. 1964. Osteology and morphology of the genera *Cristivomer* and *Salvelinus* and their relationship with other Salmonidae. Ph.D. Thesis. Univ. Ottawa, Ottawa, Ont. 296 p.

1968. Morphology and taxonomy of the aurora charr, *Salvelinus fontinalis timagamiensis*. Nat. Mus. Can. Bull. 237: 18 p.

Reshetnikov, Yu, S., and K. A. Savvaitova. 1962. Some peculiarities of structure of the skull and of the tail region of the skeleton, in charrs of the genus *Salvelinus*. Vopr. Ikhtiol. 2: 433–440. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 796, 1966)

Richardson, J. 1836. Fauna Boreali Americana III. Richard Benthley, London. 327 p.

Savvaitova, K. A. 1961. Intraspecific biological forms of *Salvelinus alpinus* (L) in Kamchatka. Vopr. Ikhthiol. 1: 695–706. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 795, 1966)

Steel, B. G., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to biological sciences. McGraw-Hill Book Inc., New York, N.Y. 481 p.

Tchernavin, V. V. 1923. An attempt towards a systematic arrangement of certain Salmonoidei, based on osteological characters. Izv. Inst. Opytoni Agron. (Petrograd) 1: 103–106.

1937. Skulls of salmon and trout. Salmon Trout Mag. 88: 235–242.

Vladykov, V. D. 1954. Taxonomic characters of the eastern North American charrs (*Salvelinus* and *Cristivomer*). J. Fish. Res. Board Can. 11: 904–932.

1957. Les formes locales de la truite rouge du Québec (*Salvelinus marstoni*). Nat. Can. 84(12): 233–248.

1962. Osteological studies on Pacific salmon of the genus *Oncorhynchus*. Bull. Fish. Res. Board Can. 136: 1–172.

1963. A review of salmonid genera and their broad geographical distribution. Trans. R. Soc. Can. (Ser. IV) 1: 459–504.

Waters, C. A. 1960. A study of the life history and taxonomic position of the blueback trout, *Salvelinus oquassa* (Girard). M.Sc. Thesis. Univ. Maine, Orono, Me. 90 p.

Lobster Nutrition: The Effect on *Homarus americanus* of Dietary Protein Levels

J. D. Castell and S. D. Budson

Department of the Environment
Fisheries and Marine Service, Halifax Laboratory, Halifax, N.S.

Castell, J. D., and S. D. Budson. 1974. Lobster nutrition: the effect on *Homarus americanus* of dietary protein levels. J. Fish. Res. Board Can. 31: 1363–1370.

Adult American lobsters (*Homarus americanus*) were fed artificial diets ranging between 0 and 60% protein and made isocaloric by adjusting the content of cornstarch. Those fed the highest level of protein were in the best condition; each decrease in protein resulted in a poorer condition of the lobsters. When lobsters were fed diets at the maintenance level of 0.5% of the body weight in food per day, decreasing the dietary protein content resulted in increasing weight loss and decreasing molt incidence, serum protein content, percent edible meat, heart weight, hepatopancreas and gonad weights, and increased moisture content of several tissues. Although the serum glucose level was dependent upon the cornstarch content of the diet, the glycogen content of the hepatopancreas was not affected by dietary starch levels. The glycogen content of the hepatopancreas showed a very large range of values when protein was omitted from the diet.

A relationship between serum calcium level and molt cycle was demonstrated. Serum calcium values went from an average of 50 mg% after molting to over 70 mg% 1 mo before molting.

Castell, J. D., and S. D. Budson. 1974. Lobster nutrition: the effect on *Homarus americanus* of dietary protein levels. J. Fish. Res. Board Can. 31: 1363–1370.

Nous avons donné à des homards americains (*Homarus americanus*) des régimes artificiels s'échelonnant de 0 à 60% en protéines et rendus isocaloriques par réglage de la teneur en amidon de maïs. Les homards soumis au niveau le plus élevé en protéines sont en meilleure condition; chaque diminution de niveau résulte en une condition plus pauvre. Lorsque des homards sont nourris de régimes au niveau de subsistance de 0.5% du poids corporel de nourriture par jour, une diminution du contenu en protéines dans le régime entraîne un accroissement de perte de poids et une diminution de la fréquence de mue, du contenu en protéines sériques, du pourcentage de chair comestible, du poids du coeur, du poids de l'hépatopancréas et des gonades, et une augmentation de la teneur en eau de plusieurs tissus. Bien que le niveau du glucose sérique dépende de la teneur en amidon de maïs du régime, le contenu en glycogène de l'hépatopancréas n'est pas affecté par les niveaux d'amidon du régime. Le contenu en glycogène de l'hépatopancréas montre une gamme étendue de valeurs quand les protéines sont omises du régime.

Nous démontrons une relation entre le niveau du calcium sérique et le cycle de la mue. Les valeurs du calcium sérique vont de 50 mg% après la mue à plus de 70 mg% un mois avant la mue.

Received December 20, 1973
Accepted May 2, 1974

Reçu le 20 décembre, 1973
Accepté le 2 mai, 1974

The increasing demand for lobsters (Genus *Homarus*) has resulted in an extremely high market value for them and provoked considerable interest in the possibility of farming lobsters. As with other cultured species, success in rearing lobsters will be dependent upon a precise knowledge of the animals' requirements, particularly the nutritional requirements. Although lobsters have been held in captivity for extensive periods, i.e. from egg to sexually mature specimens (Hughes and Matthiessen 1962), little is known of the dietary requirements.

In our laboratory, preliminary feeding trials with lobsters have shown that a number of physiological parameters vary directly with food supply, e.g. hemolymph serum protein and nonprotein nitrogen; hepatopancreatic glycogen, lipid, and moisture content, and weight, and hence are possible indicators of the adequacy of experimental diets (Stewart et al. 1972). An additional index was provided

Printed in Canada (J3253)
Imprimé au Canada (J3253)

by Stewart et al. (1967) who showed previously a highly significant correlation between serum protein concentration and the amount of muscle present in lobster chelae and tail.

This initial report describes the effects, on lobster growth and several physiological parameters of feeding isocaloric artificial diets containing, 0, 20, 40, and 60% casein. This report and the continuing study of the American lobster's (*Homarus americanus*) nutritional requirements have the double goal of providing information on which to base formulated commercial feeds for farming ventures and of contributing to the understanding and management of natural lobster populations upon which the present lobster industry depends.

Materials and Methods

Adult American lobsters (*H. americanus*), weighing between 300 and 500 g, and with their chelae immobilized by rubber bands, were obtained locally in November 1971. Five lobsters were distributed randomly among each of 15 137-liter fiberglass tanks, which were supplied with approximately 2 liters/min continuously flowing, filtered sea water at 20 C. This temperature was chosen as Hughes et al. (1972) had shown that lobsters not only survive at 22–24 C but also grow at a rate 4 times as fast as at normal sea temperatures.

Diets were prepared according to the formulations listed in Table 1. Dry ingredients (40 parts by weight) were thoroughly mixed with hot water (60 parts by volume) and refrigerated until the agar had set. The cheese-like solid blocks were then cut into pieces approximately 0.5 × 0.5 × 5 cm and stored frozen in plastic bags. All diets were prepared fresh each month and fed at a rate of 0.5% of the body weight of the lobsters as moist feed per day, 7 days a week. This was a maintenance feeding level for lobsters receiving Diet 5 containing 60% protein in previous unreported feeding trials.

Each lobster was weighed and a 5-ml hemolymph sample taken initially and again at regular monthly intervals throughout the experiment. All were held unfed for 1 mo before commencing feeding trials. Each artificial test diet was fed to triplicate groups of five lobsters. The lobsters were starved for 24 h prior to each monthly weighing, and 5 ml of hemolymph was drawn at that time from the ventral abdominal sinus of each animal and allowed to clot. The clot was broken up and the serum prepared by centrifugation (Stewart et al. 1966).

Serum protein was estimated using the Technicon® Auto Analyzer II and the biuret reaction as outlined in the Technicon® Procedure AA II-14. Bovine serum albumin supplied by Nutritional Biochemicals Co. was used as the calibrating standard.

Serum calcium was estimated by the method of Gitelman (1967). The automated analysis procedure was slightly modified as the high content of protein in lobster serum was coagulated by the reagents and caused blockage of the tubing. The serum was mixed with an equal volume of 0.3 N HCl containing 2.5 g/liter 8-hydroxyquinoline, and the precipitated protein was removed by centrifugation in a clinical centrifuge. The supernatant fluid was then processed on the AutoAnalyzer as outlined in Technicon® Procedure AA II-03.

The automated glucose oxidase–peroxidase procedure of Hill et al. (1961) was used to estimate the low levels of glucose typical of lobster serum. The glucose was dialyzed into a 1.61% Na_2SO_4 solution containing 0.5% Triton-X. The enzyme reagent contained 1.5 g glucose oxidase (30,000 units/g Nutritional Biochemicals) per liter of 0.1 N, pH 7 phosphate buffer. The hydrogen peroxide produced from the enzymatic reaction was detected as the chromatophore of 0-dianisidine measured at 460 mμ. The Technicon® Alkaline Phosphatase Manifold was used with the AutoAnalyzer II for the automated enzymatic analysis of glucose.

TERMINATION

At the end of the 4-mo feeding trial, the lobsters were anesthetized with isobutyl alcohol (Foley et al. 1966). The dorsal portion of each carapace was cut away to permit removal of the heart, gonad, hepatopancreas, and a portion of the dorsal muscle from the first anterior tail segment. The animals were then boiled in sea water

TABLE 1. Composition of artificial diets used in protein level study.

	% dry weight in diet number				
Ingredient	1	2	3	4	5
Casein	Starved	0.0	20	40	60
Cornstarch		65	45	25	5
Cod liver oil		10	10	10	10
Alpha-cellulose		15	15	15	15
Mineral mix[a]		3	3	3	3
Vitamin mix[b]		2	2	2	2
70% choline[a] chloride		1	1	1	1
Vitamin E acetate		0.2	0.2	0.2	0.2–
Agar		3.8	3.8	3.8	3.8

[a] Bernhart-Tormelli salt mix from Nutritional Biochemicals Co.

[b] Vitamin mix: thiamin (HCl) 0.32%, riboflavin 0.72%, niacinamide 2.56%, biotin 0.008%, Ca-pantothenate 1.44%, pyridoxine (HCl) 0.24%, folic acid 0.96%, menadione 0.08%, cobalamine 0.266%, i-inositol (meso) 12.50%, ascorbic acid 6.00%, p-aminobenzoic acid 2.00%, vitamin D_2 (850,000 USP/g) 0.0245%, vitamin A (500 IU/g) 0.50%, BHA 0.075%, celite 73.2343%. This vitamin mix was based on that used for rainbow trout (*Salmo gairdneri*) (Castell et al. 1972).

for 15 min and the edible meat determined as a percentage of the live weight according to Stewart et al. (1967). Water content of the gonad, hepatopancreas, and muscle was estimated by drying tissue portions at 110 C for 24 h. Glycogen content of the hepatopancreas was determined with the phenol-sulphuric acid reagent (Montgomery 1957), after digestion in 30% KOH.

Results

At the end of the 4-mo feeding period, the lobsters tended to lose weight as protein content in the diet increased. None of the starved lobsters molted during the experiment (Fig. 1). The number of molts per treatment increased with increasing dietary protein from 0 to 40% casein. Although the lobsters receiving 60% casein in their diet had fewer molts than those receiving 40%, the number of premolt lobsters on the highest protein diet was greater than for any other treatment.

Accidental deaths confused the mortality data. Cannibalism and "gas-bubble disease"[1] accounted for 50% or 5 of the 10 lobsters which died during the experiment (Table 2).

Average initial serum protein content of the serum was about 4.5% which usually decreased with decreasing protein content of the diet (Fig. 2).

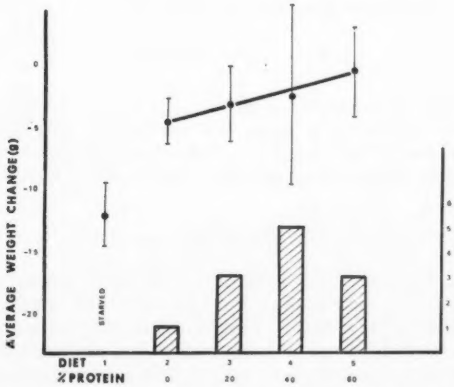


FIG. 1. The effect of dietary protein level on average weight change of unmolted lobsters (with 95% confidence limits based on standard error of the mean) and incidence of molting. Each point represents the average of 15 animals. Feeding period: 4 mo. Initial average weights were 453, 450, 474, 464, and 453 for diets 1, 2, 3, 4, 5.

TABLE 2. Mortality data for American lobsters (*Homarus americanus*) during 4-mo feeding.

Diet	Total mortality/diet	Diet induced[a] mortality/diet	Treatment
1	3	3	Starved
2	2	1	0% Protein
3	1	0	20% Protein
4	3	1	40% Protein
5	1	0	60% Protein

[a] Not due to cannibalism or gas-bubble disease.

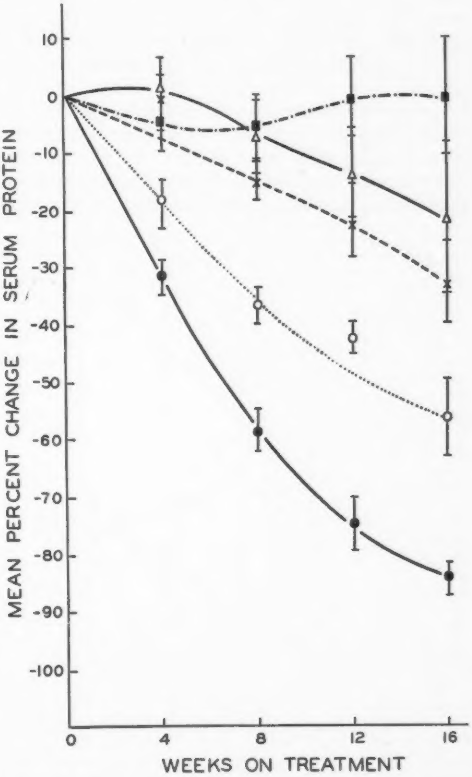


FIG. 2. The effect of dietary protein level on serum protein levels over a 4-mo feeding period, showing 95% confidence limits on the means. The mean initial serum protein values with 95% confidence limits were 4.56 ± 0.60, 4.14 ± 1.49, 5.13 ± 1.32, 4.22 ± 1.35, and 5.19 ± 1.18 for diets 1–5, respectively. Other data as in Fig. 1.
●——● Diet 1, ○·····○ Diet 2, ×---× Diet 3, △——△ Diet 4, ■-·-·-■ Diet 5.

---

[1] Cold sea water was heated to 20 C in a closed pressurized heat exchanger resulting in water supersaturated with air. Several mortalities occurred as a result of a failure of the degassing mechanism.

The group receiving 60% protein lost no serum protein over the 16-wk feeding period.

Increasing dietary protein levels tended to yield more edible meat (Fig. 3).

The changes in serum calcium (Fig. 4A) did not show a linear relationship with dietary protein level. The starved lobsters had the lowest serum calcium and the animals fed on the 60% protein diet the highest at the end of 16 wk. As in the case of live weight and serum protein, the initial mean serum calcium values of lobsters were not the same for each group. Although the average trends are somewhat confusing, individual lobsters showed a trend towards increases in serum calcium levels to over 70 mg% before molting, followed by a rapid drop to about 50 mg% shortly after molt (Fig. 4B). One lobster on Diet 5, sampled within several hours after it molted, had a serum calcium level of 113 mg%.

The serum glucose levels at 4 wk (Fig. 5) were related inversely to the dietary protein level but directly related to the dietary cornstarch. The glycogen content of the hepatopancreas (Fig. 6) was similar for the animals on diets 3, 4, and 5. Except in the extreme case of Diet 2 (no protein), the amount of starch in the food had no effect on glycogen deposition in the hepatopancreas. The average deposition for Diet 2 was higher but extreme variability among individuals on this diet produced results not significantly different (5% level) from those on other diets. The starved lobsters, as expected, had low hepatopancreatic glycogen contents.

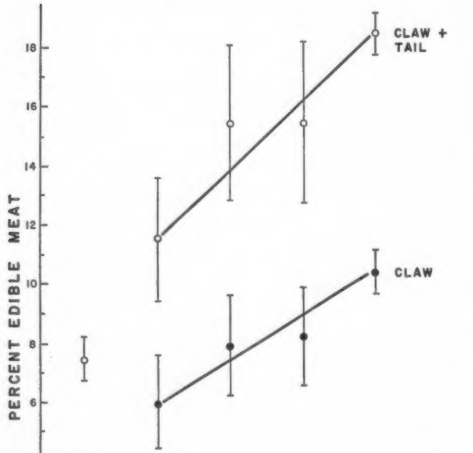


FIG. 3. Differences in percent of the total live body weight represented by edible meat in claw and tail caused by altered dietary protein levels. Vertical bars represent 95% confidence limits based on standard error of the mean. Other data as in Fig. 1.

The percentages of the body weight represented by the heart (Fig. 7), gonad (Fig. 8) and hepatopancreas (Fig. 9) all tended to increase with increasing dietary protein levels. The percentage of body weight represented by the heart showed a linear increase between 0 and 40% dietary protein but no difference between 40 and 60% protein (Fig. 7). The gonad and hepatopancreas did not show this levelling-off effect.

The mean moisture content with 95% confidence limits for hepatopancreas, gonad, and dorsal muscle are given in Fig. 10. The effect of dietary protein level on moisture content is most pronounced in the hepatopancreas and least significant in muscle tissue. The moisture content of the female gonad is much more strongly affected by dietary protein than the male organ. Most of the females on diets 1 and 2 had reabsorbed their eggs. The gonads were straw colored, emaciated, and watery in appearance. The females receiving 20% or more protein had dark green ovaries. One female on each of the diets (diets 3, 4, and 5) receiving protein had become gravid before the end of the feeding trial. The data for percent edible meat and serum protein level were submitted to linear regression analysis and the following equation was obtained

$$Y = 7.38 + 2.42 (X)$$

where X is percent protein in the serum and Y is the percent edible meat. The correlation coefficient R was 0.9085 showing a definite relationship between serum protein and meat content of the lobsters.

Discussion

The lobsters fed artificial diets in this study have shown a requirement for high levels of dietary protein. In terms of physical measurements such as weight change and organ size and biochemical measurements such as serum protein and hepatopancreas glycogen, each increase in dietary protein resulted in an improved condition of the lobsters. There are at least two possible explanations for the high dietary protein requirement of lobsters. It is possible that one or more essential amino acids in casein are limiting. Casein is generally considered to be an excellent source of essential amino acids for mammals such as rats and is used as the sole protein in many nutrition studies for this reason. The essential amino acid requirements are not the same for all species; for example, due to the high requirement for glycine by chicks their diet would have to contain 300% casein to meet this demand (Block and Weiss 1956). As nothing is known of the amino acid requirements of lobsters, the possibility of a limiting essential amino acid in casein cannot be ruled out at present. The essential amino acid requirements of the lobster will be a topic of future work at this laboratory.

A second possible explanation for the results of the present feeding study, is that the lobster's metabolism is such that it requires high dietary levels of protein. Considerable evidence exists suggesting that this latter explanation is the correct one. In its natural environment the lobster is a carnivore, feeding on animals such as crabs, sea urchins, starfish, and molluscs (Weiss 1971), which are relatively high in protein. The gastric juices of lobsters have been found to contain higher levels of protease activity than any other type of digestive enzyme (Brockerhoff et al. 1970). Hoyle (1973) was unable to increase the amylase activity in the digestive juice of lobsters by feeding various levels of dietary cornstarch; the feeding, however, did increase the protease activity. The fact that lobsters have relatively low levels of serum glucose (4–35 mg% compared to 90–200 mg% for humans) and high levels of nonprotein nitrogen (Stewart et al. 1972), suggests that they utilize protein as an energy source as well as for tissue formation.

Other marine decapod crustaceans have also shown high demand sfor protein. Kitabayashi et al. (1971) found that the Kuruma prawn exhibited the best growth and survival on test diets with high protein content. The protein level was varied by adding increasing amounts of squid meal included in a mixture of squid meat extract, squid liver extract, vitamin mix, and other added nutrients. Kanazawa et al. (1970), using more refined nutrients in their artificial diets, showed that 50% or more soybean protein resulted in the fastest growth of Kuruma prawns. Forster and Beard (1973) found that the prawn *Palaemon serratus* exhibited a similar growth-increasing characteristic for protein up to 70% in the diet. It thus seems probable that carniv-

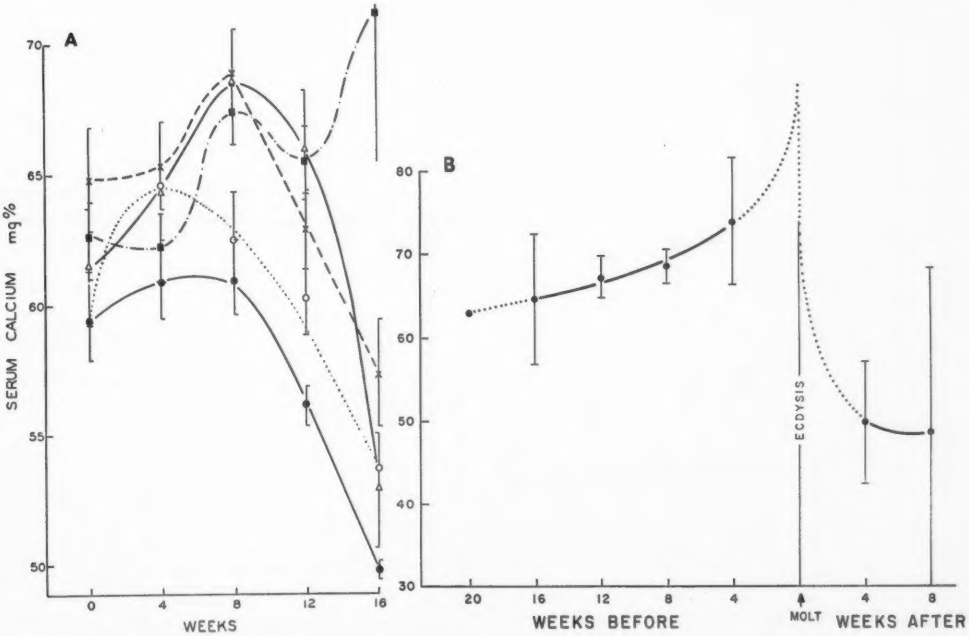


FIG. 4. A) Variations in mean serum calcium content of lobsters on each dietary treatment over the 4-mo feeding period. Vertical bars represent 95% confidence limits of the means. ●——● Diet 1, ○·····○ Diet 2, ×---× Diet 3, △——△ Diet 4, ■-·-·-■ Diet 5. B) Relationship between serum calcium and weeks before and after molt.

orous, marine, decapod crustaceans in general have a requirement for high levels of dietary protein.

In addition to the nutritional requirement results of this experiment, there are many interesting physiological results. The mortalities were surprisingly low, even among the lobsters starved at 20 C for 5 mo. The three lobsters on Diet 1 and one on Diet 2 (Table 2) which died for reasons other than cannibalism or gas-bubble disease had serum protein levels of less than 0.6% on the monthly sample taken prior to their deaths. All four of these lobsters had initial serum protein values of less than 2%, whereas all of those surviving starvation or the 0% protein diet had initial serum protein values of greater than 2%, confirming the contention of Stewart et al. (1969) that serum protein content is a good indicator of physical condition in lobsters.

Stewart et al. (1967) have shown that a good correlation exists between the serum protein concentration and the percent edible meat. The relationship was described by the following equations:

$$Y = 15.65 + 1.54\ (X) \text{ for wild lobsters and}$$

$$Y = 11.65 + 2.15\ (X) \text{ for captive fed and starved lobsters}$$

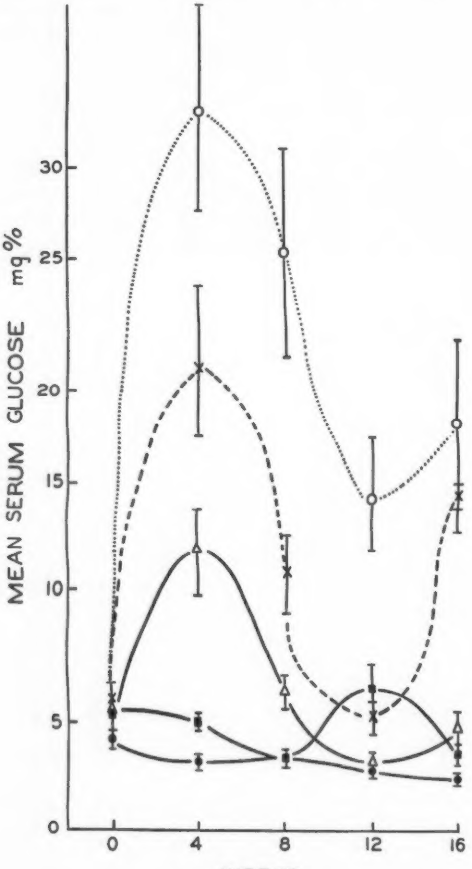


FIG. 5. Variations in mean serum glucose content for lobsters on each dietary treatment over the 4-mo feeding period. Vertical bars represent 95% confidence limits on the means.
●——● Diet 1, ○⋯⋯○ Diet 2, ×‒‒‒× Diet 3, △——△ Diet 4, ■——■ Diet 5.

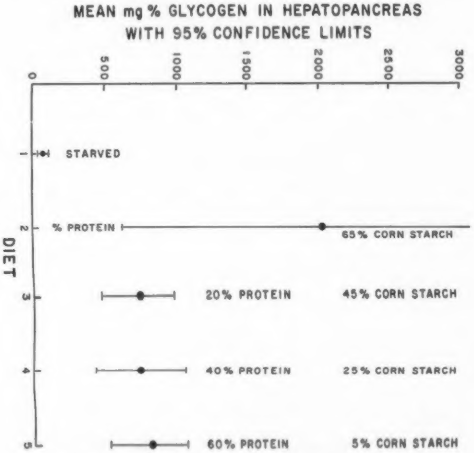


FIG. 6. Effect of diets on glycogen content of lobster hepatopancreas.

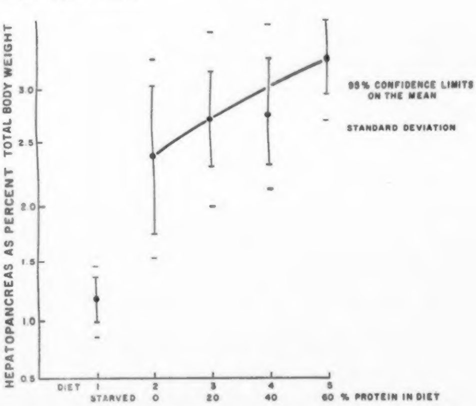


FIG. 7. Effect of dietary protein on heart weight as indicated by percent of the total body weight represented by the heart.

Where X is percent protein in the serum and Y is the percent edible meat in the claw and tail. Linear regression analysis of the data from the present feeding study resulted in a similar regression line with $R = 0.9085$, showing an extremely good correlation. These linear expressions varied considerably depending on the condition of the lobsters used in the analysis. The average serum protein content of the wild lobsters was much higher than the average value for the captive fed and starved lobsters analyzed by Stewart et al. (1967). The average serum protein value of the lobsters used from our artificial diet study was even lower than the average of either the fed or starved group. As the average serum protein content of the lobsters used for the regression analysis decreased, the Y intercept decreased and the dependence of Y on X increased, suggesting a nonlinear relationship.

Another important result from the serum analysis is the relationship between molt cycle and serum calcium (Fig. 4B). This result is consistent with that of Travis (1955) who reported a good correlation between serum calcium and molt cycle for the spiny lobster *Panulirus argus*. The high average

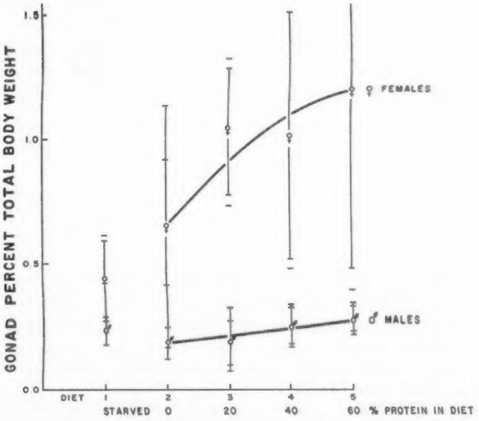


FIG. 8. Effect of dietary protein level on weight of male and female sex organs as indicated by the percent of the total body weight represented by the gonad.

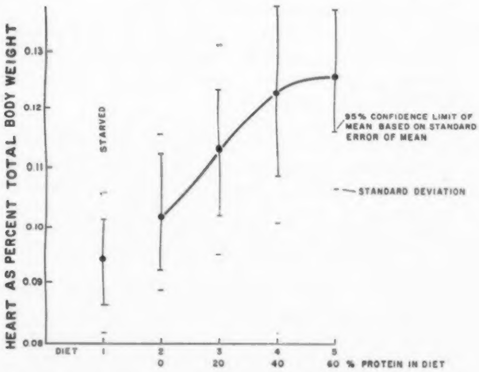


FIG. 9. Effect of dietary protein level on hepatopancreas weight as indicated by the percent of the total body weight represented by this organ.

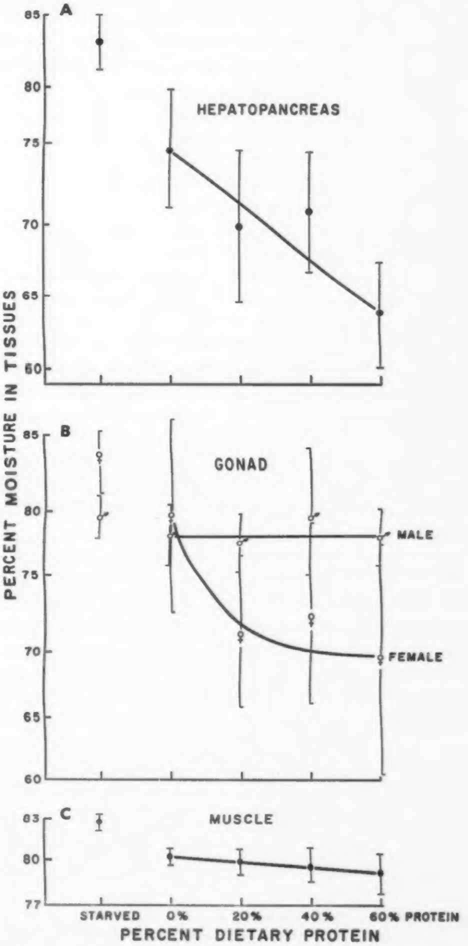


FIG. 10. Effect of dietary protein level on moisture content of: A) hepatopancreas, B) gonad, and C) muscle tissue.

serum calcium of Diet 5 lobsters (60% protein, Fig. 4A) thus indicates a large number of premolt individuals. At termination of feeding Diet 5 animals had shown a lower incidence of molting than Diet 4 animals (40% protein). The serum calcium data suggest that had the feeding been continued for a few more months there would have been a more positive relationship between molt incidence and dietary protein content, with Diet 5 animals having the highest number of molts.

The molt cycle of lobsters adds a complicating factor to the study of their nutrition which does not exist for fish and agricultural animals. Whereas most animals have a fairly continuous growth curve, lobsters and other crustaceans have a stepwise growth pattern. It is important in interpreting analytical data for lobsters that the stage in the molt cycle be considered. A good example of this was seen in the percent edible meat data (Fig. 3). The average value for Diet 4 lobsters is lower than a linear relationship would indicate. These lobsters had the highest incidence of molt, but the percentage of edible meat is low in newly molted lobsters. The Diet 3 lobsters had a higher percent edible meat value than the straight line in Fig. 3 would indicate. Figure 4A shows that Diet 3 lobsters had the second highest levels of serum calcium and thus, a higher percentage of premolt lobsters.

The results of these experiments have shown that the large variation in condition indices resulting from changes through the molt cycle add an extra dimension of difficulty to lobster nutrition studies. This may be overcome in one of three ways. The first is through the use of large numbers of animals per treatment to overcome individual variations. The second possible solution is to run paired comparison experiments matching animals by weight, stage in molt cycle, and general condition as judged by serum protein and calcium values. The third solution is to use very long-term feeding experiments so that dietary differences become much more significant than does individual variation. Future work will involve consideration of these mediating factors.

Block, R. J., and K. W. Weiss. 1956. Amino acid handbook methods and results of protein analysis. Charles C. Thomas, Springfield, Ill. 161 p.

Brockerhoff, H., R. J. Hoyle, and P. C. Hwang. 1970. Digestive enzymes of the American lobster (*Homarus americanus*). J. Fish. Res. Board Can. 27: 1357–1370.

Castell, J. D., et al. 1972. Essential fatty acids in the diet of rainbow trout (***Salmo gairdneri***): growth, feed convension and some gross deficiency symptoms. J. Nutr. 102: 77–85.

Epifanio, C. 1973. Crabs thrive on new feed. Shellfish Soundings XXXV: 14.

Foley, D. M., J. E. Stewart, and R. A. Holley. 1966. Isobutyl alcohol and methyl pentynol as general anesthetics for the lobster, *Homarus americanus*) Milne-Edwards. Can. J. Zool. 44: 141–143.

Forster, J. R. M., and T. W. Beard. 1973. Growth experiments with the prawn (*Palaemon serratus*) Pennant fed with fresh and compounded foods. Fish. Invest. 27(7): 1–16.

Gitelman, H. J. 1967. An improved automated procedure for the determination of calcium in biological specimens. Anal. Biochem. XVIII: 521.

Hill, J. B., G. Kessler, and N. Y. Chauncey. 1961. An automated determination of glucose utilizing a glucose oxidase–peroxidase system. J. Lab. Clin. Med. 57: 970–980.

Hoyle, R. J. 1973. Digestive enzyme secretion after dietary variation in the lobster (*Homarus americanus*). J. Fish. Res. Board Can. 31: 1647–1653.

Hughes, J. T., and G. C. Matthiessen. 1962. Observations on the biology of the American lobster, (*Homarus americanus*). Limnol. Oceanogr. 7: 414–420.

Hughes, J. T., J. J. Sullivan, and R. Shleser. 1972. Enhancement of lobster growth. Science 177: 1110–1111.

Kanazawa, A., M. Shimaya, M. Kawasaki, and K. Kashiwada. 1970. Nutritional requirement of prawn I. Feeding on artificial diets. Bull. Jap. Soc. Sci. Fish. 36: 949–954.

Kitabayashi, K., K. Shudo, K. Nakamara, and S. Ishawa. 1971. Studies of formula feed for Kuruma prawn. V. On the growth-promoting effects of protein level in a diet and re-examination of ingredients used. Bull. Tokai Reg. Fish. Res. Lab. Tokyo No. 65: 139–147.

Montgomery, R. 1957. Determination of glycogen. Arch. Biochem. Biophys. 67: 378–386.

Stewart, J. E., J. W. Cornick, D. M. Foley, M. F. Li, and C. M. Bishop. 1967. Muscle weight relationship to serum protein, hemocytes, and hepatopancreas in the lobster, (*Homarus americanus*). J. Fish. Res. Board Can. 24: 2339–2354.

Stewart, J. E., J. R. Dingle, and P. H. Odense. 1966. Constituents of the hemolymph of the lobster, (*Homarus americanus*) Milne-Edwards. Can. J. Biochem. 44: 1447–1459.

Stewart, J. E., G. W. Horner, and B. Arie. 1972. Effects of temperature, food, and starvation on several physiological parameters of the lobster (*Homarus americanus*). J. Fish. Res. Board Can. 29: 439–442.

Stewart, J. E., and M. F. Li. 1969. A study of lobster (*Homarus americanus*) ecology using serum protein concentrations as an index. Can. J. Zool. 47: 21–24.

Travis, D. F. 1935. The molting cycle of the spiny lobster, ***Panulirus argus*** Latreille. III. Physiological changes which occur in the blood and urine during the normal molting cycle. Biol. Bull. 109: 484–503.

Weiss, H. M. 1971. The diet and feeding behaviour of the lobster, (*Homarus americanus*) in Long Island Sound. Ph.D. Thesis. Univ. of Connecticut, Storrs, Conn.

Pathophysiology of Infectious Hematopoietic Necrosis Virus Disease in Rainbow Trout (*Salmo gairdneri*): Early Changes in Blood and Aspects of the Immune Response after Injection of IHN Virus[1]

DONALD F. AMEND

U.S. Fish and Wildlife Service, Bureau of Sport Fisheries
and Wildlife
Western Fish Disease Laboratory, Seattle, Wash. 98115, USA

AND LYNNWOOD SMITH

College of Fisheries
University of Washington, Seattle, Wash. 98115, USA

AMEND, D. F., AND L. SMITH. 1974. Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout (*Salmo gairdneri*): early changes in blood and aspects of the immune response after injection of IHN virus. J. Fish. Res. Board Can. 31: 1371–1378.

Juvenile rainbow trout (*Salmo gairdneri*) were injected with infectious hematopoietic necrosis (IHN) virus and various hematological and blood chemical changes were monitored over 9 days. The packed cell volume, hemoglobin, red blood cell count, and plasma bicarbonate were significantly depressed by day 4. Plasma chloride, calcium, phosphorus, total protein, and blood cell types did not change during the 9 days. Furthermore, plasma $B_4^{2\prime}$ LDH isozyme was significantly increased by the fourth day, and fish infected with infectious pancreatic necrosis virus, *Vibrio anguillarum*, *Aeromonas salmonicida*, and redmouth bacterium did not show specific LDH isozyme alterations. Acid-base alterations occurred at 10 C but not at 18 C. The acid-base imbalance and elevation of the $B_4^{2\prime}$ LDH isozyme were consistently associated with the early development of the disease.

The immune response after injection of IHN virus was determined and protection from disease was tested by passive immunization. Actively immunized fish developed IHN-neutralizing antibodies within 54 days after injection of virus, and the antibodies were protective when juvenile fish were passively immunized and experimentally challenged with IHN virus.

AMEND, D. F., AND L. SMITH. 1974. Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout (*Salmo gairdneri*): early changes in blood and aspects of the immune response after injection of IHN virus. J. Fish. Res. Board Can. 31: 1371–1378.

Nous avons injecté le virus de la nécrose hématopoïétique infectieuse (IHN) dans de jeunes truites arc-en-ciel (*Salmo gairdneri*) et avons suivi de façon continue, pendant 9 jours, les divers changements hématologiques et chimiques du sang. Le volume d'hématies concentrées, l'hémoglobine, le compte de globules rouges du sang et la teneur en bicarbonate du plasma sont nettement diminués au jour 4. Le chlorure plasmique, calcium, phosphore, protéines totales et types de cellules sanguines ne changent pas durant la période de 9 jours. De plus, il y a augmentation significative, au 4e jour, de l'isozyme $B_4^{2\prime}$ de la LDH du plasma, et les poissons infectés du virus de la nécrose pancréatique infectieuse, *Vibrio anguillarum*, *Aeromonas salmonicida* et bactérie de la bouche rouge ne montrent pas de modifications spécifiques de l'isozyme de la LDH. L'équilibre acide-base change à 10 C mais non à 18 C.

---

[1] This paper reports a portion of the work encompassed by a thesis submitted to the University of Washington School of Fisheries in partial fulfilment of the requirements for a Ph.D. degree.

Printed in Canada (J3332)
Imprimé au Canada (J3332)

Le déséquilibre acide-base et l'augmentation de l'isozyme $B_4^{2'}$ de la LDH sont uniformément associés au premier développement de la maladie.

Nous avons déterminé la réponse immunologique après injection du virus de l'IHN et vérifié, par immunisation passive, la résistance à la maladie. Les poissons activement immunisés développent des anticorps contre IHN en dedans de 50 jours après injection du virus, et les anticorps offrent une protection aux jeunes poissons immunisés passivement et exposés expérimentalement au virus de l'IHN.

Received February 26, 1974
Accepted May 8, 1974

Reçu le 26 février, 1974
Accepté le 8 mai, 1974

PHYSIOLOGICAL changes associated with disease are used extensively in mammalian medicine for diagnostic purposes and to determine the prognosis of disease. Several attempts have been made to determine the physiological status of fish infected with various pathogens (Hunn 1964; Bell 1968; Wedemeyer and Ross 1973; Mulcahy 1969; Field et al. 1944; Foda 1973; Cardwell and Smith 1971) and with some toxic chemicals (McKim et al. 1970; Wedemeyer 1971). However, most physiological tests are not routinely used in fisheries medicine because an insufficient number of diseases have been studied for comparative purposes and because normal values have not been widely established or the tests standardized. The recent works of McCarthy et al. (1973) and Wedemeyer and Nelson (1974) have aided much in establishing the normal ranges of many physiological parameters of rainbow trout (*Salmo gairdneri*).

Amend (1973) described the physiological status of moribund rainbow trout infected with infectious hematopoietic necrosis (IHN) virus. The disease was characterized by a severe depletion of the alkali reserve and an imbalance of blood electrolytes resulting in decreased blood osmolality. These changes may have been caused by a loss of renal function because of the viral infection.

This paper describes some of the early physiological changes following experimental injection of IHN virus; compares infected fish at two water temperatures; compares plasma lactic dehydrogenase (LDH) isozymes of IHN-infected fish with the LDH isozymes of some bacterial diseases; and sets forth a preliminary description of the immune response of rainbow trout to IHN virus.

Materials and Methods

EXPERIMENTAL PROCEDURES

Four series of tests were conducted. In the first test about 600 rainbow trout 10–13 cm long were placed in each of two 700-liter circular tanks containing 9 C flowing municipal water. Chemical characteristics of the water are described elsewhere (Amend et al. 1969). After fish were acclimated for 2 wk, all fish in one tank were anesthetized with 50 ppm Benzocain and injected intraperitoneally (ip) with 0.1 ml of IHN virus preparation containing $10^{6.4}$ $TCID_{50}$/ml grown in fathead minnow cells (FHM). The fish in the other tank were handled similarly except they received only 0.1 ml ip of cell culture medium. Fish were randomly sampled 24 h after inoculation and sampling continued at 24-h intervals for 9 days. The fish were not fed during the experiment.

At each sampling period, 40 fish were anesthetized, the caudal peduncle was severed, and blood was collected in heparinized capillary tubes. Precautions were taken to prevent hemolysis. From 10 fish, a red blood cell count (RBC), packed cell volume (PCV), hemoglobin (Hb), and blood smear were taken. A capillary tube of blood from each of these 10 fish was plugged for plasma bicarbonate analysis. All capillary tubes were centrifuged at 1000 × *g* for 10 min.; then the plasma from the 30 unplugged tubes was pooled into three 10-fish groups and frozen at –20 C for other biochemical analyses.

In the second test 14 rainbow trout (20–28 cm) were placed in each of four 75-liter troughs. Two of the four troughs received 10 C flowing water and the other two troughs received 18 C water. The fish in one trough at each temperature were injected with 0.1 ml ip of IHN virus preparation ($10^{6.6}$ $TCID_{50}$/ml) and the other fish received 0.1 ml ip of cell culture media. After 6 days all fish were anesthetized and the blood from each fish was collected in heparinized capillary tubes and sealed. Plasma pH, bicarbonate, chloride, and LDH were determined on each fish.

In the third test, the plasma LDH isozyme patterns were compared from rainbow trout (8–9 cm) infected with *Vibrio anguillarum*, *Aeromonas salmonicida*, and redmouth (RM) bacterium (Hagerman strain). Ten fish were injected ip with 0.1 ml of a standardized bacterial suspension for each pathogen and held at 15 C. The bacteria were grown on trypticase soy agar (plus 1% NaCl for *Vibrio*) for 24 h at 20 C, then harvested and washed once with saline, and adjusted to 40% transmittance at 625 mμ. Ten control fish were injected with saline. Blood was collected as described above when signs of disease were evident within 24–72 h post injection. The 10 control fish were bled 72 h after injection. Plasma was also obtained from moribund 6–7 cm brook trout (*Salvelinus fontinalis*) naturally infected with infectious pancreatic necrosis (IPN) virus. Plasma from IHN-infected rainbow trout from a previous test was used for comparison.

The objective of the fourth test was to determine if rainbow trout formed antibodies to IHN virus and to determine if these antibodies would confer protection from the disease by passive immunization. Five adult rainbow trout (about 500 g each) were actively immunized by injecting intravenously 1 ml of cell-culture-grown (rainbow trout embryonic) IHN virus ($10^{6.5}$ $TCID_{50}$/ml). Five control fish in a separate tank received 1 ml of cell culture media from noninfected cells. All fish were held in flowing 8 C water for 5 days and then at 17 C for 21 days. The temperature was again lowered to 8 C and the fish were given a second injection. After 5 days the temperature was again raised to 17 C, where it remained for the remainder of the test. Each fish was fin-clipped with a different mark for identification. Serum samples were taken at 0, 26, 54, and 90 days after the first injection, next complement inactivated at 45 C for 30 min, and then tested for neutralization against IHN virus.

The rationale for the above procedure is that animals respond to live viruses better than to killed viruses (Fenner and White 1970). Rainbow trout are readily infected at 8 C, but the disease does not develop at 17 C (Amend 1970). By injecting the fish at a low temperature, a viremia is established, but the infection stops before death occurs. Furthermore, the immune response is faster at 17 C. This procedure optimizes the antigen dose and the antibody response.

Adsorption tests were performed by adding an equal volume of cell-culture-grown IHN virus ($10^{5.0}$ $TCID_{50}$/ml) serum diluted 1:20 at 15 C for 1 h; then the virus was inactivated at 45 C for 1 h. The serum was centrifuged at 30,000 × *g* to sediment the virus, and then the serum retested for viral neutralization.

To test if the virus-neutralizing ability of the serum would confer protection against the disease, the serum from three immunized fish was pooled. Fifteen rainbow trout fingerlings (5–6 cm) were passively immunized by injecting 0.1 ml ip of undiluted immune serum, and the same number of control fish were injected with nonimmune serum. Additional controls consisted of nonchallenged and IHN-challenged fish which received no serum. All fish, except the nonchallenged controls, were injected subcutaneously anterior to the mid-dorsal line with 0.01 ml of IHN virus preparation ($10^{6.6}$ $TCID_{50}$/ml). All tests were duplicated and mortalities counted for 20 days.

BIOCHEMICAL TEST

The Hb, RBC, PCV, plasma bicarbonate, and pH were determined immediately on fresh blood. Blood smears were prepared, fixed in methanol, and stained by the Lieshman–Giemsa method as described by Klontz and Smith (1968) and the cells classified as described by Klontz (1972). Plasma chlorides, calcium, phosphorus, protein, and LDH were determined on frozen samples. Methods of analyses were as follows: chloride and protein (Wedemeyer and Chatterton 1970); Hb, PCV, bicarbonate, and pH (Wedemeyer and Chatterton 1971). Calcium was determined by titration, phosphorus by the Molybdate Method, and RBC with a coulter counter. Horizontal starch gel electrophoresis was used for LDH analysis and the isozymes were identified according to Utter and Hodgins (1972). The pCO_2 and H_2CO_3 concentrations were determined mathematically using the Henderson-Hasselbalch equation for blood carbonate.

VIROLOGICAL TESTS

A permanent cell line of FHM and an established cell line of rainbow trout embryonic (RTE) cells were used. Eagle's minimal essential medium (Earl's salts) containing 10% agamma newborn calf serum, 100 μg per ml penicillin G, and 100 μg per ml of streptomycin was used in all tests. IHN virus was from a laboratory stock (1274) originally isolated from rainbow trout and was injected into fish to test for virulence. The reisolated virus was specifically neutralized by anti-IHN serum. Viral preparations were grown at 15 C until all cells showed cytopathic effects, 4–6 days after inoculation. All preparations were centrifuged at 1000 × *g* for 10 min to sediment cellular debris. The method of Reed and Meunch was used to determine viral titers and all titrations were performed in microtiter plates. Serum for neutralization tests was preabsorbed with cells and media before the test was conducted. Twofold dilutions of serum against a constant 50–100 $TCID_{50}$ of IHN virus was used in the neutralization tests.

Results

HEMATOLOGICAL AND BIOCHEMICAL CHANGES

In a preliminary test to determine the susceptibility of the test fish to the disease, only 42% of the fish died; mortality was highest 9 days after injection. Consequently, during subsequent tests mortality was expected to range between 40 and 60%. In the first experiment, signs of the disease were noted (fecal casts) 4 days after injection and the first mortality occurred on the sixth day. Mortalities increased considerably on the eighth day. Because we were interested only in the early changes associated with the disease, the experiment was terminated on the ninth day.

Samples of control and infected fish were taken daily. Consequently, at the termination of the experiment, there were about 90 individual analyses on fresh blood, and 27 pooled samples representing 270 fish on the frozen samples from both test groups. The data were then tested by analysis of variance. Because no significant differences ($P = .05$) were found within the control values for each analytical test, the composite 9 day mean and standard deviation were determined and used as representative of the population. These values were: RBC ($\times 10^6$/ml) = 1.300 ± 0.158, PCV (%) = 46 ± 5, Hb (g/100 ml) = 9.2 ± 0.8, calcium (mg/100 ml) = 10.1 ± 0.4, phosphorus (mg/100 ml) = 13.3 ± 0.6, protein (g/100 ml) 5.6 ± 0.3, bicarbonate (mEq/liter) = 10.2 ± 2.5, and chloride (mEq/liter) = 120 ± 7. The *t*-test was then used to analyze daily means of the infected fish.

Significant reduction in PCV, Hb, RBC, and bicarbonate occurred on the fourth or fifth day which was at least 24 h before the first mortality (Fig. 1). The changes were most evident by the severe depletion of the serum bicarbonate. No significant differences, however, were observed in the daily change in plasma chloride (Fig. 1), phosphorus, calcium, or total protein (Fig. 2). Furthermore, there was no significant change in the percentages of immature RBC, total leukocytes, monocytes, lymphocytes, or neutrophils (Table 1). The $B_4^{2'}$ plasma LDH isozyme was increased on the fourth day and remained elevated (Fig. 3).

Some physiological changes could be detected before the onset of mortalities, and serum bicarbonate and LDH levels were most obviously affected. Reduction in serum bicarbonate suggests alteration in the acid–base regulation. A test was then conducted to determine if acid–base and LDH changes occur at both 10 and 18 C. At 10 C the plasma bicarbonate was not significantly changed, but significant reduction ($P = .05$) of pCO_2 and an increase in plasma pH and HCO_3^-/H_2CO_3 ratio had occurred (Table 2). At 18 C there was no significant change between control and infected fish. The acid-base alteration as indicated in Fig. 1 was confirmed, but occurred only at the lower temperature.

The results of the LDH tests were similar; the $B_4^{2'}$ LDH isozyme increased in 10 of 13 infected fish at 10 C, but did not change in an equal number of fish at 18 C or in any of the control fish. Plasma

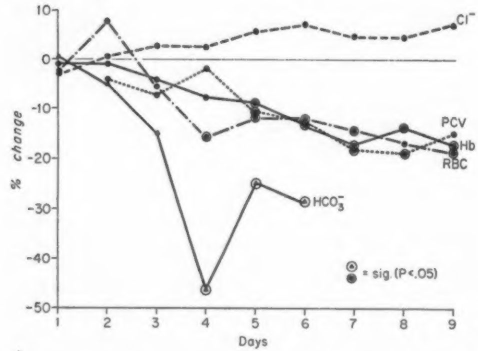


FIG. 1. Daily chloride, packed cell volume, hemoglobin, red blood cell count, and bicarbonate values from IHN infected rainbow trout (*Salmo gairdneri*) expressed as the percentage change from mean values of the uninfected population.

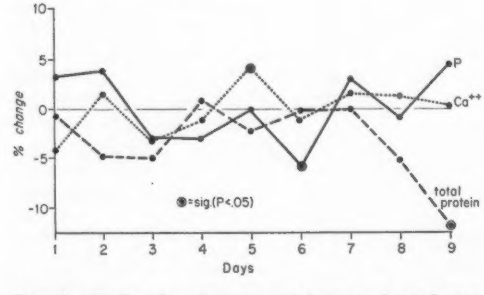


FIG. 2. Daily phosphorous, calcium, and total protein values from IHN infected rainbow trout expressed as the percentage change from mean values of the uninfected population.

TABLE 1. Hematological comparison of blood smears from uninfected controls and from rainbow trout (*Salmo gairdneri*) infected with IHN virus.[a]

Cell type	Control Mean ± SE	IHN infected Mean ± SE	P[b]
Immature RBC			
Day 1	9.2±0.9	8.2±1.0	NS
Day 5	7.0±1.0	8.0±0.7	NS
Day 9	6.9±0.9	10.3±1.9	NS
Leukocytes (Total)			
Day 1	0.9±0.2	1.5±0.3	NS
Day 2	2.0±0.3	1.9±0.6	NS
Day 9	1.9±0.3	3.4±2.3	NS
Monocytes			
Day 1	3.3±0.8	2.3±1.0	NS
Day 5	4.8±1.0	4.0±1.3	NS
Day 9	5.8±1.8	6.2±1.6	NS
Lymphocytes			
Day 1	91.2±1.6	89.8±3.0	NS
Day 5	93.0±1.0	92.8±2.2	NS
Day 9	88.4±3.2	91.8±2.9	NS
Neutrophils			
Day 1	5.6±1.6	7.9±2.6	NS
Day 5	2.2±0.8	3.2±1.1	NS
Day 9	5.8±1.7	2.0±1.6	NS

[a] Immature RBC and leukocytes are percentages of about 500 cells counted. including mature RBC. Monocytes, lymphocytes, and neutrophils are differential percentages of about 100 leukocytes counted. Ten fish were used in each comparison.

[b] NS = not significant.

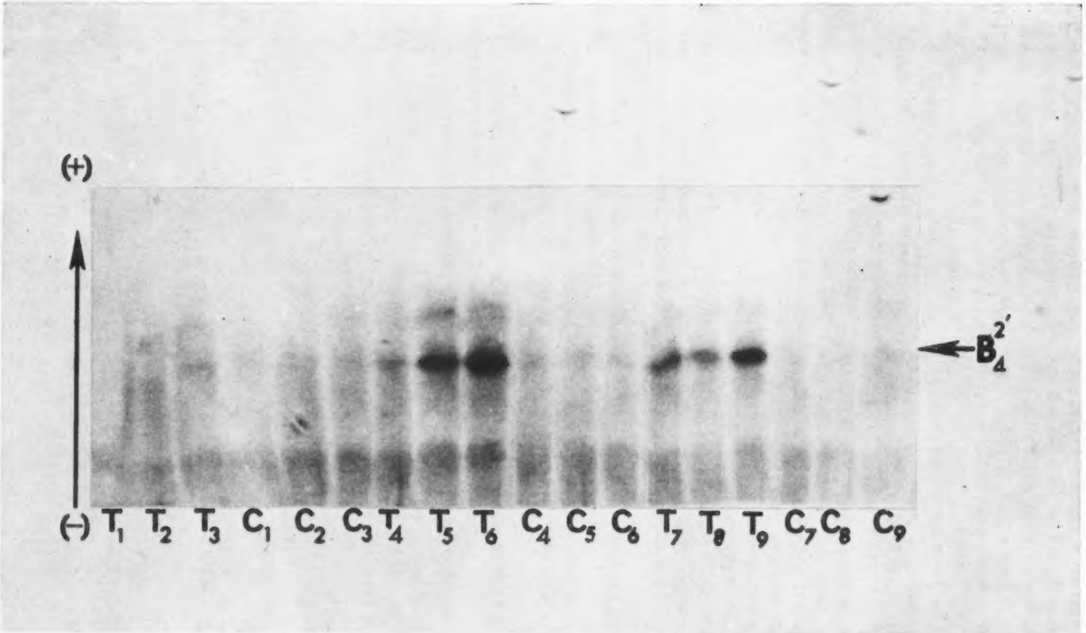

(+)
(−)
T_1
T_2
T_3
C_1
C_2
C_3
T_4
T_5
T_6
C_4
C_5
C_6
T_7
T_8
T_9
C_7
C_8
C_9
$B_4^{2'}$

FIG. 3. Starch–gel electrophoresis of plasma LDH isozymes from infected (T) rainbow trout (*Salmo gairdneri*) and uninfected controls (C). Subscript indicates days following injection. Arrow indicates location of $B_4^{2'}$ LDH isozyme.

Amend and Smith — J. Fish. Res. Board Can.

LDH isozyme patterns were then compared on moribund fish infected with *A. salmonicida*, *V. anguillarum*, RM bacterium, and IPN virus to determine if the $B_4^{2\prime}$ LDH change was specific for IHN. In all cases the disease was confirmed by reisolation of the agent, and with IPN, histological examination and serum neutralization confirmation were also performed. The number of samples showing increased LDH on the $B_4^{2\prime}$ isozyme was as follows: IPN — 0/10, RM — 0/5, *A. salmonicida* — 1/7, *V. anguillarum* — 1/7. The one fish with elevated $B_4^{2\prime}$ LDH in *A. salmonicida* and *V. anguillarum* also had an increase in the slower migrating isozymes. With IHN, only the $B_4^{2\prime}$ isozyme was elevated. Therefore, of the five diseases compared, only IHN could be distinguished by the LDH isozyme pattern.

ASPECTS OF IMMUNOLOGY

Adult rainbow trout were injected with a live virus preparation and the temperature was manipulated to prevent disease. Sera were then tested for viral neutralization. All sera, including those from control fish, were toxic to cell cultures at a dilution of 1:20 or less; therefore, the lowest dilution recorded was 1:40. Bleeding prior to injection of virus revealed no detectable anti-IHN activity (Table 3). No antiviral activity was detected 26 days after injection, but four of five fish had significant IHN-neutralizing antibody by day 54. The results were only slightly changed on day 90. These sera neutralized the IHN virus, but had no activity against IPN virus. Furthermore, absorption of the sera with IHN virus completely removed the antiviral activity. The above results are characteristic of specific antibody formation and not interferon (Dekinklin and Dorson 1973).

The above data show that rainbow trout can be immunized against IHN virus, and in other tests, trout which survived a natural epizootic showed anti-IHN neutralization (unpublished data). To demonstrate whether the antibody was protective, the sera of three fish were pooled (average 50% neutralizing titer 1:320). It was injected into fingerling rainbow trout, and then the fish were challenged with virulent virus. None of the passively immunized fish died, but an average 55% of the fish receiving control serum died (Table 4). These data indicate that the antibody formed by rainbow trout against IHN disease is protective.

Discussion

Anemia is a characteristic sign of IHN disease during natural epizootics (Amend et al. 1969),

TABLE 2. Comparison of acid-base balance between uninfected control and IHN infected rainbow trout at two water temperatures.

Clinical Test[a]	Number	Control mean ± SE	Number	Infected mean ± SE	P[b]
		10 C			
pH	11	7.508±0.030	13	7.599±0.038	*
HCO_3^- (MEq/L)	13	11.4 ±0.3	14	11.0 ±0.5	NS
pCO_2 (mmHg)	10	15.87 ±0.81	12	12.69 ±1.38	*
H_2CO (mEq/L)	10	0.476±0.024	12	0.381±0.041	*
HCO_3^-/H_2CO_3	10	25.31 ±1.8	12	30.9 ±2.0	*
Total CO_2(MEq/L)	10	12.18 ±0.33	12	11.40 ±0.58	NS
		18 C			
pH	6	7.454±0.013	14	7.485±0.023	NS
HCO_3^- (mEq/L)	6	11.7 ±0.5	14	11.2 ±0.5	NS
pCO_2 (mmHg)	6	17.30 ±0.94	14	15.58 ±0.84	NS
H_2CO_3 (mEq/L)	6	0.519±0.028	14	0.468±0.025	NS
HCO_3^-/H_2CO_3	6	22.7 ±0.7	14	24.7 ±1.3	NS
Total CO_2	6	12.22 ±0.53	14	11.71 ±0.56	NS

[a] pCO_2, H_2CO_3, HCO_3^-/H_2CO_3, and total CO_2 are calculated values and were derived by the formula $\text{pH} = 6.1 \text{ Log } \frac{HCO_3^-}{.03\ pCO_2}$ which is valid only at 38 C. These data, except for pH, are not temperature corrected and are not to be taken as normal values, but are included for comparative purposes because all data and fish were handled similarly.

[b] * = ($P = 0.05$), NS = not significant.

but severe anemia may or may not occur during experimental infections or during acute natural epizootics. Anemia, though helpful as a diagnostic aid, is not a specific sign of any disease. Numerous diseases and conditions can cause anemia in fish. Many anemic conditions in fish have not been characterized, but Amend (1973) described IHN disease as a normocytic aplastic anemia. In the present paper we have shown that the changes in blood occurred early in the infection. Although there were significant reductions in mean blood values, they were still in the normal range and compatible with survival (Wedemeyer and Nelson 1974; McCarthy et al. 1973).

Plasma calcium, phosphorus, chloride, and total protein remained unchanged, and no detectable changes were noted in the abundance of immature RBC, total leukocytes, macrophages, lymphocytes, or neutrophils. Amend (1973) found that fish terminally infected with IHN disease had a significant depression of plasma electrolytes and a reduction of neutrophils. In the present study random samples were selected from a population in which only 50% of the fish were estimated to have contracted a fatal infection. Consequently, the data would not reflect the severe changes expected in moribund fish. More severe changes may have occurred in these tests, but were undetected because the clinical changes had not developed to the extent described by Amend (1973). Inclusion of both sick and apparently healthy fish in the analyses would explain the wide variation in the data.

Alteration in plasma $B_4^{2'}$ LDH isozyme concentrations and in acid–base balance are two changes which appear early in the infection and are consistently associated with the disease. The degree of base deficit or pH change would depend largely on the ability of the fish to compensate for acid-base changes. In terminally infected fish there were severe depletions in alkali reserve and an increase in blood pH (Amend 1973). In the present study alkali depletion was suggested in the first test, but an uncompensated alkalosis was indicated in the second test. Although bicarbonate was not significantly changed in the second test, elevated pH and elevation of the bicarbonate/carbonate ratio indicates a loss of acid (pCO_2). The specificity of the acid-base imbalance due to IHN disease is not known because we are unaware of any similar analyses with other fish diseases.

The specificity of the $B_4^{2'}$ LDH isozyme concentration changes is also not known, but it appears that similar changes do not occur with IPN, *A. salmonicida*, *V. anguillarum*, or RM disease. However, Wroblewski (1957) points out that several conditions are capable of causing an increase in serum LDH in mammals, and may not be specific. Furthermore, Utter and Hodgins (1972) reported that genetic variability of the $B_4^{2'}$ isozyme occurs in the serum of rainbow trout. It is not known what change might occur in fish without this isozyme.

TABLE 3. Serum neutralization titer of rainbow trout immunized against IHN virus. Sera were tested against 50–100 $TCID_{50}$ IHN virus.

	Reciprocal of 100% neutralization titer			
	Days			
Fish	0	26	54	90
1	<40	<40	>320	>160
2	<40	<40	160	<80
3	<40	<40	<40	<40
4	<40	<40	not tested	1280
5	<40	<40	160	320
Controls (5 fish)	<40	–	–	<40

TABLE 4. Passive immunization of fingerling rainbow trout by ip injection of undiluted sera from adult rainbow trout actively immunized with IHN virus. Fingerlings were challenged by subcutaneous injection of $10^{6.6}$ $TCID_{50}$/ml of IHN virus at 10 C and mortalities counted for 20 days.

	Control (no virus)		Control (virus)		Antiserum		Serum control	
	A	B	A	B	A	B	A	B
No. dead	0	0	13	6	0	0	9	7
No. alive	15	15	1	9	12	12	5	8
Total	15	15	14	15	12	12	14	15
% dead	0	0	93	40	0	0	64	47
Combined	0/30		19/29		0/24		16/29	
% average	0		66		0		55	

Elevating the water temperature from 10 to 18 C prevented disease in IHN-infected sockeye salmon (Amend 1970). Preliminary tests also indicated that the disease would also not develop in rainbow trout at 18 C (unpublished data). However, it was not known in either experiment if the fish were uninfected or if they only experienced a subclinical infection which did not result in death. The data presented here indicate that the infection was terminated at the higher temperatures. The acid–base balance did not change, nor did the $B_4^{2\prime}$ LDH levels increase, which was characteristic early in the infection at lower temperatures. DeKinklin and Dorson (1973) demonstrated the presence of an interferon at 18 C in rainbow trout infected with viral hemorrhagic septicema (VHS) but only weakly at lower temperatures. Whether this or some other physiological explanation is responsible for the temperature effect is not known for IHN disease.

The immune response of fish to viral infections is just beginning to be understood. Klontz et al. (1965) suggested that certain protein changes in the serum macroglobins may be of immunological significance in sockeye salmon following infection with IHN virus. Wolf and Quimby (1969) reported that rainbow trout which were injected with IPN virus developed IPN-neutralizing antibodies. Dorson (1972) also found that rainbow trout were capable of responding immunologically to bacteriophages. However, Jørgensen (1971) was not able to demonstrate antibody in rainbow trout naturally infected with VHS virus, but was able to demonstrate neutralizing antibodies in artificially infected fish only after 18 mo post injection. IHN resembles VHS in many ways, but in this test rainbow trout responded very well to IHN virus. Perhaps it was the virulent virus and temperature manipulation that optimized the immunological response.

Passive immunization is one way of demonstrating the relative importance of cellular immunity and humoral antibody in conferring protection from disease. The cellular immune response in mammals is sometimes important in combating initial viral infections and may even be responsible for some of the signs of the disease (Fenner and White 1970). However, humoral antibody is most important for preventing second attacks from the same disease. The results presented here demonstrate that humoral anti-IHN antibody in rainbow trout can prevent fatal infection of IHN disease.

The ability to control viral infections in fish must depend on a thorough understanding of the disease. Knowledge of the physiological changes can help determine what environmental, diet, or gas regulation should be tested to help alleviate the disease. Chemotherapeutics are useless to combat viral infections; therefore, we must rely on environmental control, immunization, or eradication of the agent. Eradication is not always practical. Environmental control is being practiced and as diseases become better understood, may become more widely practiced. Results presented here suggest that salt and acid–base regulations may influence the course of IHN disease. Immunization is the most widely utilized method of protection from viral diseases im mammalian medicine. In fact, almost all major breakthroughs in controlling viral diseases have been through the development of effective vaccines. This goal may be attainable with the fish viruses when we obtain a better understanding of the immune mechanisms of fish, the antigenic makeup of the viruses, and the diseases they cause.

Acknowledgments

We thank Drs H. Hodgins and C. Evans for the review and many helpful suggestions, and J. Pietsch and Cathy Smith for their technical help.

AMEND, D. F. 1970. Control of infectious hematopoietic necrosis virus by elevating the water temperature. J. Fish. Res. Board Can. 27: 265–270.

1973. Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout. Ph.D. Thesis. Univ. Washington, Seattle, Wash. 102 p.

AMEND, D. F., W. T. YASUTAKE, AND R. MORGAN. 1969. Some factors influencing susceptibility of rainbow trout to the acute toxicity of an ethyl mercury phosphate formulation (Timsan). Trans. Am. Fish. Soc. 98: 419–425.

BELL, G. R. 1968. Distribution of transaminases (amino transferases) in the tissues of Pacific salmon (*Oncorhynchus*), with emphasis on the properties and diagnostic use of glutaminoxalacetic transaminase. J. Fish. Res. Board Can. 25: 1247–1268.

CARDWELL, R. D., AND L. S. SMITH. 1971. Hematological manifestations of vibriosis upon juvenile chinook salmon. Prog. Fish-Cult. 33: 232–235.

DEKINKLIN, P., AND M. DORSON. 1973. Interferon production in rainbow trout (*Salmo gairdneri*) experimentally infected with egtved virus. J. Gen. Virol. 19: 125–127.

DORSEN, M. 1972. Some characteristics of antibodies in the primary immune response of rainbow trout (*Salmo gairdneri*), p. 129–140. *In* Symp. Zool. Soc. Lond. No. 30.

FENNER, F., AND D. O. WHITE. 1970. Medical virology. Academic Press, New York, N.Y. 390 p.

FIELD, J. B., L. L. GEE, C. A. ELVEHJEM, AND C. JUDAY. 1944. The blood picture in furunculosis induced by *Bacterium salmonicida* in fish. Arch. Biochem. 3: 277–284.

FODA, A. 1973. Changes in hematocrit and hemoglobin in Atlantic salmon (*Salmo salar*) as a result of furunculosis disease. J. Fish. Res. Board Can. 30: 467–468.

Hunn, J. 1964. Some patho-physiologic effects of kidney disease in brook trout. Proc. Soc. Exp. Biol. Med. 117: 383–385.

Jørgensen, P. E. V. 1971. Egtved virus: demonstration of neutralizing antibodies in serum from artificially infected rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 28: 875–877.

Klontz, G. W. 1972. Hematological techniques and the immune response in rainbow trout, p. 89–99. *In* Symp. Zool. Soc. Lond. No. 30.

Klontz, G. W., and L. S. Smith. 1968. Methods of using fish as biological research subjects, p. 323–385. *In* Methods of animal experimentation. Vol. III. Academic Press, New York, N.Y.

Klontz, G. W., W. T. Yasutake, and T. J. Parisot. 1965. Virus diseases of Salmonidae in Western United States. N.Y. Acad. Sci. 126: 531–542.

McCarthy, D. H., J. P. Stevenson, and M. S. Roberts. 1973. Some blood parameters of rainbow trout (*Salmo gairdneri* Richardson). J. Fish. Biol. 5: 1–8.

McKim, J. M., G. M. Christensen, and E. P. Hunt. 1970. Changes in the blood of brook trout (*Salvelinus fontinalis*) after short term and long term exposure to copper. J. Fish. Res. Board Can. 27: 1883–1889.

Mulcahy, M. F. 1969. Serum protein changes in U.D.N. — infected Atlantic salmon. A possible method of diagnosis. J. Fish. Biol. 1: 333–338.

Utter, F. M., and H. O. Hodgins. 1972. Biochemical genetic variations at six loci in four stocks of rainbow trout. Trans. Am. Fish. Soc. 101: 494–507.

Wedemeyer, G. 1971. The stress of formalin treatments in rainbow trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 28: 1899–1904.

Wedemeyer, G., and K. Chatterton. 1970. Some blood chemistry values for the rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 27: 1162–1164.

1971. Some blood chemistry values for juvenile coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 28: 606–608.

Wedemeyer, G., and N. C. Nelson. 1974. Effect of statistical method on blood chemistry normal range estimates for Shasta rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. (In press)

Wedemeyer, G., and A. J. Ross. 1973. Nutritional factors in the biochemical pathology of corynebacterial kidney disease in coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 30: 2696–2698.

Wolf, K., and M. C. Quimby. 1969. Infectious pancreatic necrosis: clinical and immune response of adult trouts to inoculation with live virus. J. Fish. Res. Board Can. 26: 2511–2516.

Wroblewski, F. 1957. The clinical significance of alterations in lactic dehydrogenase activity of body fluids. Am. J. Med. Sci. 234: 301–312.

Gravel Incubators: a Second Evaluation on Pink Salmon, *Oncorhynchus gorbuscha*, Including Adult Returns

ROBERT A. BAMS

Department of the Environment, Fisheries and Marine Service
Pacific Biological Station, Nanaimo, B.C.

BAMS, R. A. 1974. Gravel incubators: a second evaluation on pink salmon, *Oncorhynchus gorbuscha*, including adult returns. J. Fish. Res. Board Can. 31: 1379–1385.

A hatchery method designed for mass production of unfed Pacific salmon fry and utilizing a gravel medium during most of the incubation period is being evaluated on successive cycles of a stock of pink salmon, *Oncorhynchus gorbuscha*, of the Tsolum River, B.C. Possible treatment effects are studied at emergent fry and returning adult stages in artificially and naturally propagated populations. Average growth rate and, hence, efficiency of yolk conversion were unimpeded in the hatchery environment, but fry emerged 11 days prematurely. Survival from green egg to emergent fry averaged 74.9% in the hatchery and 20.6% in the creek, for a gain ratio at emergence of 3.63. Recovery of selectively marked populations of hatchery and creek fish demonstrated almost identical survival rates from fry to adult stages and a final gain ratio of 3.46. Adult lengths and weights, fecundity, and timing of migrations were unaffected generally by the hatchery treatment.

BAMS, R. A. 1974. Gravel incubators: a second evaluation on pink salmon, *Oncorhynchus gorbuscha*, including adult returns. J. Fish. Res. Board Can. 31: 1379–1385.

Une méthode de pisciculture pour la production en masse d'alevins de saumon du Pacifique privés de nourriture et qui utilise un milieu de gravier durant la majeure partie de la période d'incubation est en voie d'évaluation sur des cycles successifs d'un stock de saumons roses, *Oncorhynchus gorbuscha*, de la rivière Tsolum, C.-B. Nous étudions les effets possibles du traitement aux stades d'émergement des alevins et de retour des adultes, sur des populations propagées artificiellement et naturellement. Le rythme moyen de croissance, et partant l'efficacité de conversion du vitellus ne sont pas entravés dans le milieu de pisciculture, mais les alevins émergent 11 jours avant le temps. La survie entre le stade d'oeuf vert et celui de l'émergement des alevins est en moyenne de 74.9% en pisciculture et de 20.6% dans le ruisseau, donnant un quotient de gain à l'émergement de 3.63. La recapture de populations de pisciculture et de ruisseau marquées sélectivement démontre des taux de survie presque identiques entre les stades d'alevins et d'adultes, et un quotient de gain final de 3.46. La longueur et le poids des adultes, la fécondité et le réglage du temps des migrations ne sont en général pas affectés par le traitement en pisciculture.

Received November 19, 1973
Accepted April 30, 1974

Reçu le 19 novembre, 1973
Accepté le 30 avril, 1974

A method for artificial propagation of Pacific salmon fry was developed on the basis of increased knowledge concerning incubational requirements of larval salmon. Applications of filtered creek water and a gravel medium during incubation constitute the major improvements.

A test was designed for evaluation of the new method, comprising detailed comparisons of hatchery-raised fish with those of a naturally propagated control population. Both test populations must originate from parents comparable in genetic background, timing, and size. Treatment effects can then be evaluated at the fry stage and, in what is considered the ultimate test, the returning adult stage.

Two such tests were completed earlier and have been reported; the first one was conducted from 1967 to 1968 on pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon at Hook Nose Creek, King Island, B.C. This test was terminated at the fry stage (Bams 1970). The second test was made from 1968 to 1970 on pink salmon at Headquarters Creek, Tsolum River watershed, B.C., and was carried through to the adult stage (Bams 1972). Evaluation at the fry stage demonstrated that in both operations growth rate, developmental rate, and efficiency of yolk conversion remained un-

Printed in Canada (J3227)
Imprimé au Canada (J3227)

changed, whereas survival increased substantially and emergence from the gravel occurred at a somewhat earlier stage of development in the hatchery population. At the adult stage no significant differences were found in marine survival, size, sex ratio, fecundity, and time of migration.

This paper reports the main results of the second test in a series of three successive tests carried out at the Headquarters Creek facility. It was conducted from 1970 to 1972 on the returns of the 1968–70 operations. Details considered beyond the scope of this paper regarding facilities, methods and design, and additional or more extensive data concerning this test are available in Bams 1973a, b.

Materials and Methods

Eggs were obtained from a constant proportion of the 1970 adult pink salmon run into Headquarters Creek by retaining every sixth male and female throughout the run. Standardized spawning procedures were used and the brood was raised to the eyed-egg stage in vertical stack incubators. Live eggs were then counted electronically (accuracy >99.5%) and planted in 4 × 8 × 4-ft high (12 × 24 × 12 dm) gravel boxes equipped with an upwelling water supply and containing 3/4-in crushed rock designed to separate individual alevins and satisfy their innate righting response (Bams 1969). The remainder of the run spawned naturally in the stream above a counting weir situated at the mouth of the creek. Upon emergence from the gravel all hatchery and most creek fry were trapped daily and hand-counted; samples were obtained at regular intervals and preserved in 10% formalin. Samples were processed for individual fork lengths, in millimeters, and total wet weights, in milligrams. Statistics were calculated for samples, and for populations by weighting for abundance. A floating trap and pen upstream of the fry weir caught a proportion of the daily fry run which was used as an index to estimate daily fry output on days the weir was inoperative. Each day for 9 days equal numbers of fry from each of the two treatments were marked by excision of two fins, AdRV in hatchery fish and AdLV in creek fish. The total number of valid marks released was determined by means of a quality-control program.

Upon return from the ocean, marked fish were recovered from the commercial fishery in several fish-processing plants in Vancouver and Steveston, B.C., at a weir at the mouth of the hatchery stream, and from the river through surveys of spawned-out fish. Recovery from the fishery was less than 50% effective, at the weir 100%, and through river surveys about 30%.

Results

The adult run into Headquarters Creek in 1970 numbered 3328 fish. For the hatchery population 243 females were retained, of which 18 died unspawned. The remaining 1014 females are presumed to have spawned in the creek. Approximately 326,000 eggs were taken into the hatchery. Based on the mean fecundity established for the hatchery population 1,540,000 eggs were available for deposition in the creek. Not including eggs used for purposes other than fry production, average survivals (S) in the hatchery were from green egg to eyed egg 78.9%, from eyed egg to emerged fry 94.9%, and from green egg to emerged fry 74.9%. Total fry production from the stream was estimated at 317,500. Accuracy of the weir count can be estimated as follows. The weir actually caught 88% of the estimated total run. The remaining 12% passed through the weir during periods when the weir was inoperative, by design during both tail ends of the run, or by high water levels during April 9 and 10. Index catches by the floating trap supplied about 94% of the estimated counts, the remainder was guesswork. Allowing a liberal ±35% on all estimated counts total fence count accuracy becomes ±4.2%. Average survival in the creek was therefore 20.6 ± 0.87%, and the gain ratio (S_H:S_C) was 3.63, with a range from 3.49 to 3.80.

Creek fry started migrating earlier than hatchery fry (Fig. 1). During the central part of the runs

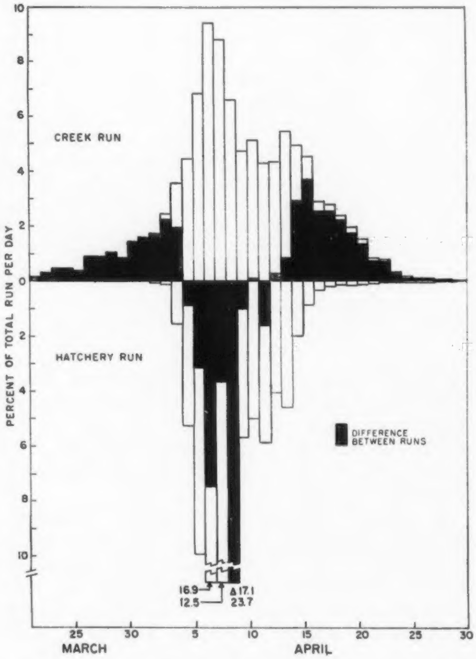


FIG. 1. Daily outputs of hatchery and creek fry in time, expressed in percent of total runs.

(20–80% points) creek fry emerged at an average of about 6% per day, hatchery fry at double that rate. Mean, median, and first and third quartile dates of the runs were as follows:

Run	Mean	SD	Quartile 1	2	3	Δ[c]
H[a]	Apr. 9.0	3.2	Apr. 6.5	8.2	9.8	0.8
C[b]	Apr. 9.5	6.4	Apr. 5.6	8.6	13.7	0.8

[a]H-Hatchery.
[b]C-Creek.
[c]Δ-Difference mean–median.

The data demonstrate that the runs had virtually identical midpoints and that the hatchery run was much more contracted than the creek run.

Fry marking took place from April 5 to 14. Exactly 104,000 validly marked fry of each source were released during this period. An additional 212,000 unmarked creek fry and 127,000 unmarked hatchery fry were released during the entire migration period.

Creek fry were longer on average than hatchery fry (Fig. 2). Total sample means (>500 fish each) differed by 0.70 mm, weighted total means by 0.55 mm. The former difference is significant, $P < 0.0001$, by Mann-Whitney U-test. Mean weights were similar in both groups (Fig. 2) and statistically indistinguishable at $P = 0.1$. Mean stage of development in nonpelagic fry is indicated by a proportionality index

$$K_D = \frac{10^3 \sqrt{W}}{L},$$

where W = larval wet weight in milligrams and L = fork length in millimeters. This index is not a condition factor, but a sensitive, size-independent indicator of relative yolk content, i.e. stage of development (Bams 1970). By this criterion development was significantly ($P < 0.0001$) less advanced in hatchery fry than in creek fry (Fig. 2). Average difference was 0.044 K_D units on total sample means and 0.048 units on weighted sample means.

Upon return from the ocean, marked test adults were recovered as follows:

Location	Origin: Hatchery	Creek	Sample size
Canneries	227	223	≈354,000
HQ weir	169	195	1,976
Surveys	13	12	858
Total	409	430	≈357,000

Based on total recoveries and original ratio at release (1.00 to 1.00) survival of marked creek fish was 2.50% better than that of marked hatchery fish from fry to adult. The difference is small and not significant ($\chi^2 = 0.526$, $P = 0.47$). Using the standard normal approximation to a binomial distribution to establish confidence limits of a proportion (e.g. Steel and Torrie 1960) which is valid with adequate sample size (for $P = 0.5$ required $n \geq 30$, our sample $n = 839$), confidence limits of the relative rates of return at the 95% level are for the hatchery fish 0.4875 ± 0.0338 and for the creek fish 0.5125 ± 0.0338. The confidence interval increases to 0.0445 at the 99% level. Hatchery effectiveness, expressed as a gain ratio, S_H: S_C, based on survivals from available eggs to returning adults was 3.46 in the marked fish populations. This compares favorably with the gain ratio obtained at the time of release, 3.63, but falls just outside the credibility range of 3.49–3.80 based on accuracy of estimate of survival in the stream.

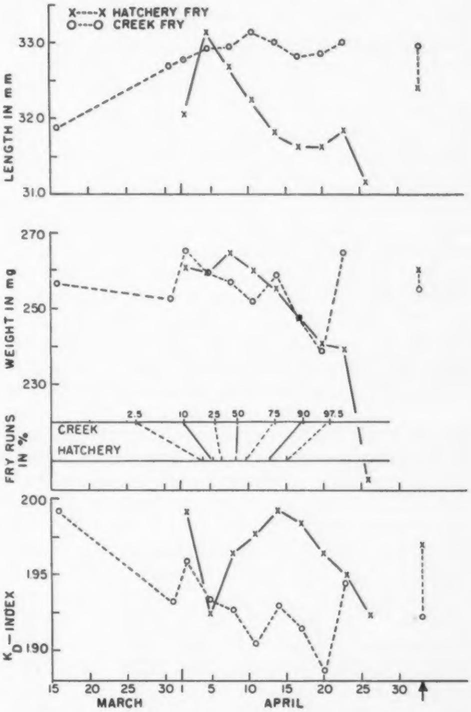


FIG. 2. Fork lengths, total wet weights, and developmental indices (K_D) of samples of hatchery and creek fry in time. Progress of the runs in time is indicated. Means weighted for abundance are indicated on the right, at ↑.

No differences between the two groups were observed in daily and cumulative records in the fishery, Fig. 3. In both groups, sex-associated differences were strongly evident: early in the run males outnumbered females, but the sex ratio gradually reversed and, despite an early closure of the fishery, more females were captured than males.

Entry into the stream showed negligible differences in mean, median, and third quartile dates of entry of the two groups (Table 1). There was a tendency among the hatchery fish for a somewhat more contracted run, Fig. 4. This is borne out by corresponding differences in variance of mean entry dates, which are significant by Barlett's test of homogeneity of variance: for the males corrected $\chi^2 = 4.55$, $P < 0.05$, and for the females $\chi^2 = 14.34$, $P < 0.001$. A similar but more pronounced contrac-

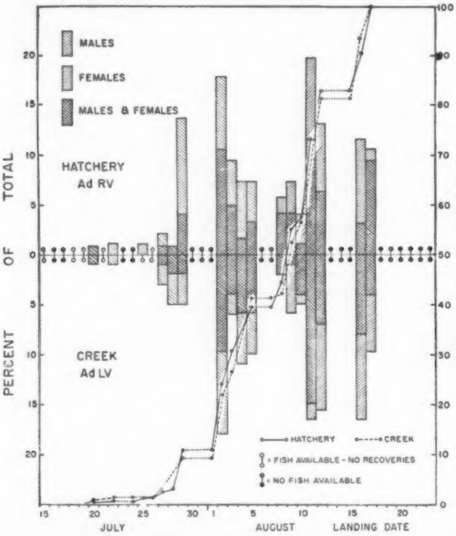


FIG. 3. Daily and cumulative recoveries of marked adult pink salmon (*Oncorhynchus gorbuscha*) of hatchery and creek origin from the commercial fishery, 1972.

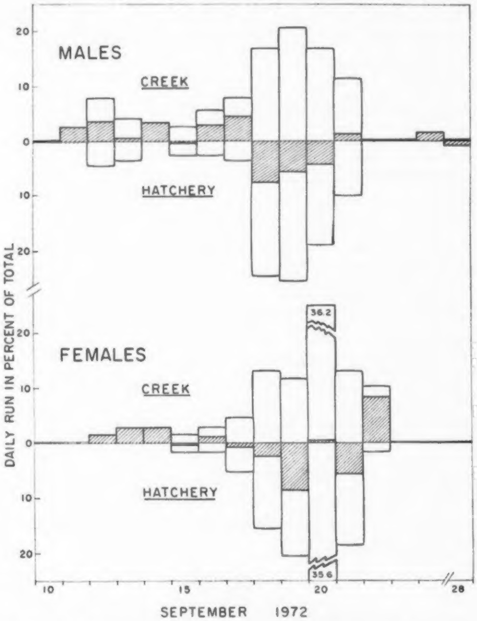


FIG. 4. Daily fence entries of marked adult pink salmon, separated by sex and incubational origin. Relative predominance indicated by cross hatching.

TABLE 1. Fence entry record for marked hatchery (AdRV), marked wild (AdLV), and unmarked (NM) pink salmon (*Oncorhynchus gorbuscha*), Headquarters Creek, 1972. Symbols: N, number of fish; SD, standard deviation; Δ (Mn–Md), difference between mean and median dates.

Source	N	Mean date	SD	Quartile 1	Quartile 2	Quartile 3	Δ (Mn–Md)
AdRV ♂	110	Sept. 18.4	2.4	17.3	18.3	19.3	+0.1
AdLV ♂	126	Sept. 17.7	2.9	15.9	18.0	19.3	–0.3
NM ♂	1006	Sept. 17.8	3.4	15.1	17.9	19.4	–0.2
AdRV ♀	59	Sept. 19.4	1.4	18.1	19.2	19.9	+0.2
AdLV ♀	69	Sept. 19.1	2.3	17.7	19.3	20.0	–0.2
NM ♀	606	Sept. 18.5	3.0	17.0	18.5	19.8	0
Total ♂♂	1242	Sept. 17.9	3.2	–	–	–	–
Total ♀♀	734	Sept. 18.6	2.8	–	–	–	–
Total	1976	Sept. 18.1	3.1	–	–	–	–

tion was observed in the hatchery fry run, as mentioned earlier. The phenomenon was not observed in the timing of the fish recovered from the fishery.

Differences in length and weight between fish of known hatchery and creek origin are shown in Table 2. Because differences were small and inconsistent in direction, no significant effect of incubational treatment on these characteristics is indicated. The same conclusion holds for variability of mean characteristics.

Returns to the hatchery stream (weir count only) consisted of 169 marked hatchery, 195 marked creek, and 1612 unmarked fish. These resulted from, respectively, 104,000 each hatchery and creek fry with double fin marks and 339,700 unmarked fry of which 37.5 ± 0.3% were of hatchery and 62.5 ± 0.3% of creek origin. The limits indicated reflect the estimated uncertainty in the fry count from the stream. Corresponding survival rates in percent were $S = 0.163$, 0.188, and 0.475 ± 0.004. When pooled the marked sources showed $S = 0.175$ and the difference in mortality between marked and unmarked fish can be expressed as $S_U/S_M = 2.71 \pm 0.02$, or $Z_M/Z_U = 1.19$.

To obtain fry-to-adult survival estimates for the total 1970–72 Headquarters Creek pink salmon population, we approximated the numbers of adults taken in the fishery as follows. Fairly good estimates are available on total numbers of pink salmon caught in the areas and during the time covered by our sampling program, the proportion of loads likely to have contained marked fish that were intercepted, and the proportions of fish inspected within loads. This information leads to the conclusion that less than one-half of the fish caught were sampled for recovery. Assuming a 100% recovery of marks on inspected fish (which is not the case), doubling the numbers of recoveries generates a conservative estimate of the totals caught. Using this estimate as a base, we estimated the number of unmarked fish of each origin by applying the ratio in survival rates established on marked and unmarked fish in the creek returns and the ratio in numbers of fry released originally. Table 3 shows known and estimated numbers of fry and adults; survival rates in percent, S, and as instantaneous rates, Z; and other statistics. In keeping with earlier calculations expressing marking mortality (e.g. Parker et al. 1963; Bams 1972) ratios of both S and Z statistics are given.

Discussion

Evaluation of the hatchery method at the fry stage established that hatchery fry were, on the average, smaller and less developed than creek fry were, but that mean times of emergence and weights were similar. To evaluate hatchery effects in terms of growth and developmental rates some adjustments of the data are necessary, because differences existed between the treatments in mean times of fertilization and in total heat units received from the water supply.

To compare growth rates and efficiency of yolk conversion in the two treatments, one may use fish lengths as an index if original mean egg sizes and stages of development at time of comparison were the same (Bams 1970). The first condition was satisfied by nonselective allocation of females to both treatments. The second condition requires a calculated conversion because at emergence the groups were not at the same mean stage of development as expressed by mean K_D values. It was established experimentally (R. A. Bams unpublished data) that at the time of maximum larval weight and at the prevailing temperatures, 0.01 K_D units

TABLE 2. Average hypural lengths in centimeters ($\bar{L}$), weights in grams ($\bar{W}$), and egg numbers ($\bar{E}$) of samples from three sources of adult pink salmon in Headquarters Creek and two sources in the fishery. Symbols: C, fish observed in creek; F, fish caught in fishery; AdRV, marked hatchery; AdLV, marked creek; NM, unmarked unknown observed in creek.

Source	N	$\bar{L}$	SD	N	$\bar{W}$	SD	N	$\bar{E}$
C. AdLV ♂	57	35.5	2.8	54	979	271	–	–
C. AdRV ♂	61	35.8	3.1	61	973	309	–	–
C. NM ♂	105	35.8	3.0	102	987	330	–	–
C. AdLV ♀	34	36.5	2.1	34	1028	196	30	1213
C. AdRV ♀	34	36.6	2.1	34	1028	199	33	1175
C. NM ♀	80	36.8	1.9	80	1065	194	64	1225
F. AdLV ♂	100	36.9	3.2	–	–	–	–	–
F. AdRV ♂	96	37.5	2.9	–	–	–	–	–
F. AdLV ♀	104	38.1	2.2	–	–	–	–	–
F. AdRV ♀	120	37.5	2.5	–	–	–	–	–

correspond with approximately 0.25 mm growth in fork length. Two estimates of the difference in mean K_D values were 0.044 and 0.048 units, which, therefore, correspond with length increments of 1.10 and 1.20 mm. This changes the observed length differentials of 0.70 and 0.55 mm in favor of the creek fry to 0.40 and 0.65 mm in favor of the hatchery fry. Therefore in the hatchery environment average growth rate from egg to fry and, hence, efficiency of yolk conversion were not inferior to those that occurred in the creek environment. Average total wet weights at emergence were similar in fry of both sources. Conversion to identical stages of development does not noticeably change the weights because fry emerged close to the stage in development at which total larval weight is at maximum and does not change over the limited range in developmental stages involved in this comparison. The present conclusions concerning growth rates and yolk conversion efficiency are in complete agreement with results obtained in earlier operations.

Mean stages of development at emergence were not the same in both treatments as indicated by the noted difference in developmental indices. At the prevailing temperatures the difference represented about 11 days' development. Therefore, relative to the creek population hatchery fry emerged at a premature stage of development equivalent to about 11 days' growth. Premature emergence from our gravel incubators has been a consistent feature in all tests to date: 1967–68 chum, 8 days and pink, 13 days; 1968–69 pink, 6 days; and 1970–71 pink, 11 days. A mean value of about 10 days seems a reasonable general estimate. The cause of this early emergence is not known, but is probably associated with fish density.

Survival in the hatchery has continued to increase with each successive operation. The present average from green egg to migrant fry of 74.9% is the highest recorded so far. However, from 80 to 90% survival to the fry stage was achieved in part of this operation and I consider such levels routinely achievable (Bams 1973a).

Survival to the fry stage was greater in the hatchery environment than in the stream. Expressed as a gain ratio (S_H: S_C) the increase was 3.63 with a credibility range from 3.49 to 3.80. Estimated gain at return was reduced to 3.46, a loss of less than 5% (range 0.8–9.4). However, a considerable net gain in production of adults was achieved. The gain ratios in the 1968–70 operation were much greater, 6.04 and 5.87, respectively (Bams 1972), which was a result of much lower creek survival (11.3% compared to the present 20.6%) and not better hatchery survival (respectively, 68.3 and 74.9%). This variability between tests demonstrates that adoption of a constant value for natural survival

TABLE 3. Survival estimates and other statistics on total estimated returns of marked and unmarked pink salmon of hatchery and creek origin.

Location and source		Marked fish		Unmarked fish
		Hatchery fish		
Catch		454		1505
Escapement		182		604
Total		636		2109
from fry	104,000	$S = 0.61$	127,200	$S = 1.66$
		$Z = 5.10$		$Z = 4.10$
		$Z_M/Z_U = 1.24$		$S_U/S_M = 2.71$
from eggs	138,800	$S = 0.46$	169,750	$S = 1.24$
		$Z = 5.39$		$Z = 4.39$
		Creek fish		
Catch		446		2469
Escapement		207		1008
Total		653		3477
from fry	104,000	$S = 0.63$	212,500	$S = 1.64$
		$Z = 5.07$		$Z = 4.11$
		$Z_M/Z_U = 1.23$		$S_U/S_M = 2.61$
from eggs	504,450	$S = 0.13$	1,030,700	$S = 0.34$
		$Z = 6.65$		$Z = 5.69$
Gain ratio S_H/S_c		3.54		3.68

would facilitate assessing the potential of the method. Gain ratios obtained in the tests are dependent on the vagaries of environmental conditions in the stream and cover far too small a number of years to be meaningful as an average. A 14-yr average value is available for survival of pink salmon in Hook Nose Creek, central B.C. (Parker 1962). This value, 7.3%, is probably representative of many coastal streams in B. C. Using this value as a base, the gain ratios in the 1968–70 and 1970–72 operations become, at the fry stage, 9.4 and 10.3, and at the adult stage, for the marked populations only, 9.1 and 9.8.

The repeatedly indicated reduction in gain ratios between the times of fry emergence and return of adults reflects, of course, the difference in survival from fry to adult in fish of the two sources, as established on the selectively marked populations. In both operations this difference in survival was insignificant ($P > 0.4$). Confidence limits were established in the text on the relative rates of return, but these can be restated in terms of differences, in percent, in survival of both sources. Expressed as survival of hatchery fish relative to creek fish we obtain for the 1968–70 operation a difference of -1.50%, ranging from -7.24 to 4.24, and for the 1970–72 operation -2.50%, ranging from -9.26 to 4.26 at the 0.95 level. These results state that, at this level of probability and as determined by the number of recoveries obtained, survival of the hatchery fish was statistically indistinguishable from that of the creek fish and that the most extreme distribution would have been a difference of less than 10% in favor of the creek fish. The original hypothesis, that the hatchery method produces fry with a survival potential equal to that of their naturally propagated counterparts, stands supported.

The third and final test in this evaluation series was initiated on the returns of the 1970–72 operation. Upon completion of the series, the general performance of the method during several years of differing environmental conditions can be estimated and compared with those of alternative propagation methods.

Acknowledgments

I thank Messrs D. G. Crabtree and R. C. Wilson for their capable execution of the often very demanding field programs, Mr J. A. C. Thomson for assistance in data processing, and Mr F. C. Withler for a critical reading of the manuscript.

BAMS, R. A. 1969. Adaptations of sockeye salmon associated with incubation in stream gravels. H. R. MacMillan Lectures on Fisheries, Symp. Salmon Trout Streams 1968: 71–87.

1970. Evaluation of a revised hatchery method tested on pink and chum salmon fry. J. Fish. Res. Board Can. 27: 1429–1452.

1972. A quantitative evaluation of survival to the adult stage and other characteristics of pink salmon (*Oncorhynchus gorbuscha*) produced by a revised hatchery method which simulates optimal natural conditions. J. Fish. Res. Board Can. 29: 1151–1167.

1973a. Evaluation of gravel incubators on first "hatchery" generation Tsolum River pink salmon, 1970–1972. Part I: Evaluation at the fry stage. Fish. Res. Board Can. Tech. Rep. 364: 18 p.

1973b. Evaluation of gravel incubators on first "hatchery" generation Tsolum River pink salmon, 1970–72. Part II: Evaluation at the adult stage. Fish. Res. Board Can. Tech. Rep. 405: 17 p.

PARKER, R. R. 1962. A concept of the dynamics of pink salmon populations, p. 203–211. *In* N. J. Wilimovsky [ed.] Symposium on pink salmon 1960. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

PARKER, R. R., E. C. BLACK, AND P. A. LARKIN. 1963. Some aspects of fish-marking mortality. Int. Comm. Northwest Atl. Fish. Spec. Publ. 4: 117–122.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. Inc., New York, N.Y. 481 p.

Fecundity and Gonad Observations of the American Eel, *Anguilla rostrata*, Migrating from Chesapeake Bay, Virginia[1,2]

CHARLES A. WENNER AND J. A. MUSICK

Virginia Institute of Marine Science
Gloucester Point, Va. 23062, USA

WENNER, C. A., AND J. A. MUSICK. 1974. Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia. J. Fish. Res. Board Can. 31: 1387–1391.

Fecundity of the American eel, *Anguilla rostrata*, was estimated from 21 specimens migrating from Chesapeake Bay during November 1970. The relationship between total length and fecundity is $\log y = -4.29514 + 3.74418 \log x$ where y is fecundity and x is total length (mm) and between total weight and fecundity is $\log y = 3.22990 + 1.1157 \log x$ where y is fecundity and x is total weight (g). Gonadal condition was described statistically and histologically. Specimens migrating from Chesapeake Bay are more sexually mature than either American eels migrating from Canadian waters or European eels, *Anguilla anguilla*, migrating from Europe. The significance of these findings with regard to Tucker's hypothesis of European eels being more sexually advanced at the onset of the spawning migration is discussed.

WENNER, C. A., AND J. A. MUSICK. 1974. Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia. J. Fish. Res. Board Can. 31: 1387–1391.

Nous avons estimé la fécondité de l'anguille d'Amérique, *Anguilla rostrata*, sur 21 spécimens émigrant de la baie Chesapeake en novembre 1970. La relation entre la longueur totale et la fécondité est la suivante: $\log y$ Œ $-4.29514 + 3.74418 \log x$, dans laquelle y est la fécondité et x la longueur totale (mm); la relation entre le poids total et la fécondité est: $\log y$ Œ $3.22990 + 1.1157 \log x$, dans laquelle y est la fécondité et x le poids total (g). Nous décrivons la condition des gonades des points de vue statistique et histologique. Les individus émigrant de la baie Chesapeake sont sexuellement plus mùrs que ceux qui émigrent des eaux canadiennes, ou que les anguilles européennes, *Anguilla anguilla*, émigrant d'Europe. Nous discutons de la signification de ces découvertes en regard de l'hypothèse de Tucker à l'effet que les anguilles européennes sont sexuellement plus avancées au moment où commence la migration de fraie.

Received August 20, 1974
Accepted April 26, 1974

Reçu le 20 août, 1973
Accepté le 26 avril, 1974

THE fall migration of the American eel (*Anguilla rostrata*) from Atlantic coast estuaries is well documented in the literature (Bigelow and Schroeder 1953; Vladykov 1955; Gray and Andrews 1970). Gray and Andrews found that migratory eels leaving Newfoundland waters were more sexually mature, with ova of a greater diameter, than European silver eels (*A. anguilla*) reported by Rasmussen (1951). Wenner (1973) found that three silver eels captured southeast of the mouth of Chesapeake Bay on December 5, 1967 were more sexually mature than Newfoundland specimens and that the gonadal condition of one specimen collected southeast of Cape Cod on November 7, 1969 was consistent with the Canadian report. He also reported the capture of six females east of Assateague Island on December 22, 1971. These last specimens were more sexually mature than the others as judged by ova diameters. These findings appear to contradict Tucker's (1959) statement that European eels are more sexually advanced at the time of migration than American eels.

Bigelow and Schroeder (1953) reported that "eels (European) are the most prolific fish, ordinary

---

[1] Adapted from part of a thesis submitted to the faculty of the College of William and Mary in partial fulfillment of the requirements of a Master of Arts degree in Marine Science by C. A. W.

[2] Contribution No. 627 from the Virginia Institute of Marine Science.

Printed in Canada (J3146)
Imprimé au Canada (J3146)

females averaging 5 to 10 million eggs, and the largest one certainly 15 to 20 million" No data were presented. Vladykov (1955) stated that female American eels have from 10 to 20 million eggs and Eales (1968) suggested that each female produces 15–20 million eggs. These reports estimate fecundity but neither relate it to fish length or weight nor provide the methodology used in obtaining the estimate.

This report describes fecundity of the American eel from the Chesapeake Bay region and gonadal condition of male and female silver eels migrating from Chesapeake Bay.

Materials and Methods

COLLECTION OF SPECIMENS

Migrating silver eels (52 males, 46 females) were obtained from commercial poundnet fishermen at Cape Charles, Va. on November 23, 1970. Specimens were transported alive to the Virginia Institute of Marine Science and were maintained at 19–22 C in a holding tank prior to being sacrificed.

HISTOLOGICAL METHODS

Animals were anesthetized in MS-222 (1 g: 1000 ml). Total length was recorded to the nearest millimeter, and total weight to the nearest gram. Gonadal tissue from 20 males and 23 females was placed in Bouin's fixative, dehydrated in a graded series of ethanol baths, embedded in Paraplast (Fisher Scientific Co.), sectioned at 8 μ on a rotary microtome, stained with Harris hematoxylin and counter-stained with eosin. Ovarian sections were viewed at 150×, 675×, and 1500×. Ten ova sectioned through the nucleus were measured with an ocular micrometer at 150× on each of 10 specimens to determine the gonadal state of the fish. Testicular sections were viewed at 675× and 1500× to determine the state of spermatogenesis.

TREATMENT OF OVA

Individual ovaries were excised from an additional 21 fish, and wet weight was recorded to the nearest 0.1 g. After 3 mo storage in Gilson's solution (Bagenal 1967) the eggs were not completely dislodged from the ovarian connective tissue. Egg clusters were completely broken up by a 3–5 min treatment with a sonic cleaner. The resultant egg suspensions were diluted to 500 or 1000 ml and three 1-ml aliquots were removed from the well-mixed suspensions. Eggs in each aliquot were counted in a gridded Sedgewick-Rafter chamber at 150×. As the eggs were slightly irregular in shape, the longest horizontal diameters of the first 50 eggs from each egg suspension were measured with an ocular micrometer at the same magnification. The maturity index (gonad weight [g], total weight [g]) was calculated for each specimen.

STATISTICAL ANALYSIS

The number of eggs and the wet weights of the left and right ovaries were compared using paired *t* tests (Sokal and Rohlf 1969). Egg diameters from the ovaries treated with Gilson's solution were compared between ovaries and among fish by a factorial design (Guenther 1964). Regression analysis was performed by an IBM 1130 computer and the 95% confidence intervals about regression were calculated according to Sokal and Rohlf (1969).

Results

DESCRIPTION OF SILVER EELS

The eels were judged to be of the silver phase because of their coloration, increased eye diameter, and gonadal condition. The lateral line was prominent, the dorsal aspect of the pectoral fin and the caudal fin were darkly pigmented and in general they fit the description of silver eels (Vladykov 1955). The arc-sine transformed values (Sokal and Rohlf 1969) of the horizontal diameter of the eye expressed as percent of the gape (Ege 1939) of 60 histologically sexed female yellow eels ($\bar{X}$ total length = 439 mm, extremes 352–687) collected from Chesapeake Bay subestuaries, silver males ($\bar{X}$ total length = 372 mm, extremes 339–438), and silver females ($\bar{X}$ total length = 633 mm, extremes 418–845) were compared by analysis of variance. Significant differences between treatment means were detected at the 99% level ($F = 169.52$, df = 2, 155). Linear contrasts performed by the Scheffé method (Guenther 1964) showed that all treatment means were significantly different from each other with silver males having the greatest eye diameter ($\bar{X} = 74.5\%$), female silver eels intermediate ($\bar{X} = 48.7\%$), and yellow eels the smallest ($\bar{X} = 42.4\%$). Macroscopic observations of the gonads showed an increase in size as compared to the gonads of yellow eels signifying the approach of sexual maturity.

FECUNDITY

Treating the egg clusters with the sonic cleaner did not alter the egg count. Tissue from an individual ovary in Gilson's solution was exposed to the sonic cleaner for 0, 3, 5, 8, and 15 min. Three 1-ml aliquots were removed from the well-mixed suspension at the end of each time period and counted to determine if the sonic cleaner destroyed a significant number of eggs. Individual egg counts were plotted against exposure time and a regression equation was calculated for the values. The slope of the equation was then evaluated by a *t* test (Sokal and Rohlf

1969) and was not significantly different from zero ($t = 0.23$, df = 13).

No significant differences existed between the left and right ovarian weights ($t = 0.84$, df = 13) and the number of eggs in the left and right ovaries ($t = 0.98$, df = 20). The values of the maturity index ranged from 0.0265 to 0.0625 with the mean and 95% confidence interval being 0.0481 ± 0.004.

Preliminary data plots showed that the relations between total length and gonad weight and total weight and gonad weight were linear over the ranges of values observed. The regressions of fecundity on total length and fecundity and total weight were curvilinear and were made linear by a logarithmic transformation. Data plots, regression relationships, and the 95% confidence belts about regression are found in Fig. 1.

An analysis of variance demonstrated a significant difference in mean ova diameter among fish ($F = 118.0$, df = 20, 2058), but not between left and right ovaries ($F = 0.33$, df = 1, 2058). Mean ova

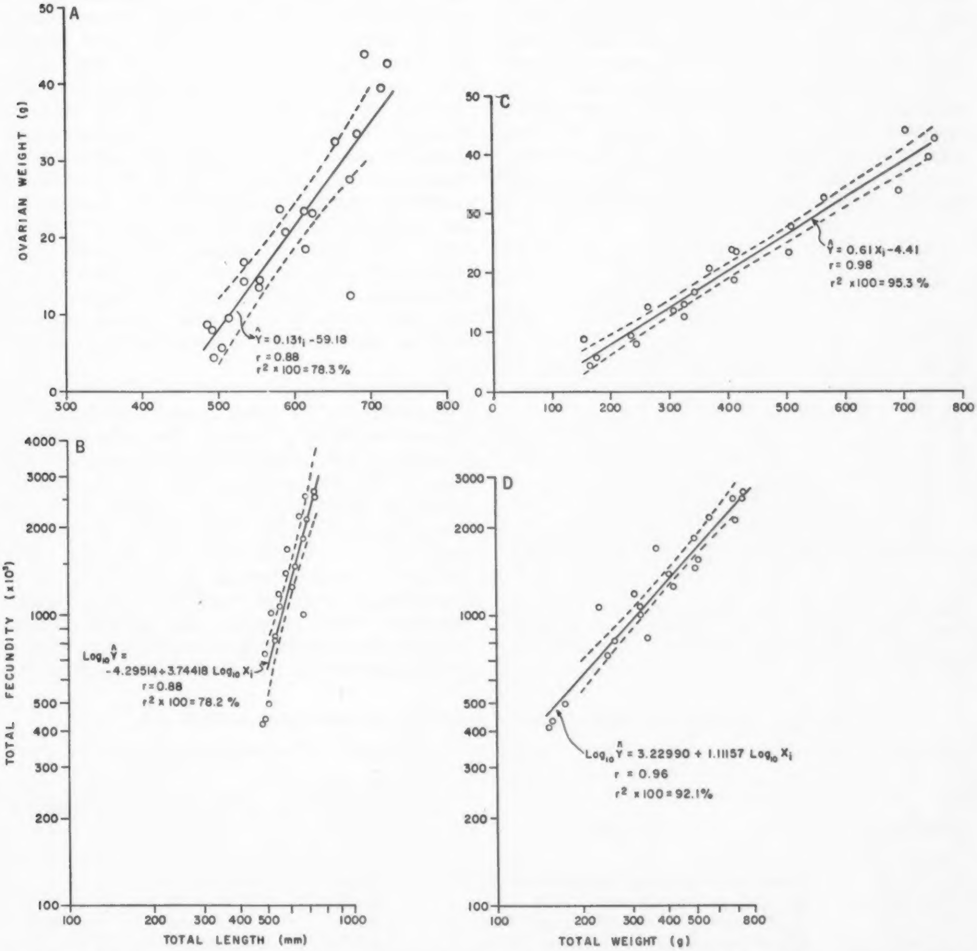


FIG. 1. Relation between: total length (mm) and A) ovarian weight (g), B) total fecundity; total weight (g) and C) ovarian weight (g), D) total fecundity, for migratory silver American eels (*Anguilla rostrato*). Each graph has the 95% confidence belt about the regression.

diameter and the 95% confidence interval from all fish was 0.244 ± 0.004 mm. The frequency distribution of the egg diameters is found in Fig. 2.

HISTOLOGY

Most eggs were spherical with a centrally located nucleus. A large number of vesicles were present in the cytoplasm, presumably representing regions of lipid concentration which were leached out during histological preparation. Extremely basophilic inclusions were observed throughout the cytoplasm in many of the larger oocytes, but they appeared more concentrated towards the periphery of the cell. These inclusions resembled yolk granules in *Brevoortia patronus* oocytes described by Combs (1969). The nuclear membrane was evident and the nucleoplasm was slightly basophilic. Deep staining basophilic inclusions were seen aound the inner side of the nuclear membrane (Fig. 3a, b, c). Developmental stages of the oocytes varied. A small fraction of the oocytes was characterized by a cuboidal shape, smaller size, and an extreme dense basophilic cytoplasm. Whether or not these cells would have developed into mature oocytes is conjecture. Testicular sections (Fig. 3d) showed nests of secondary spermatogonia as defined by Hyder (1969).

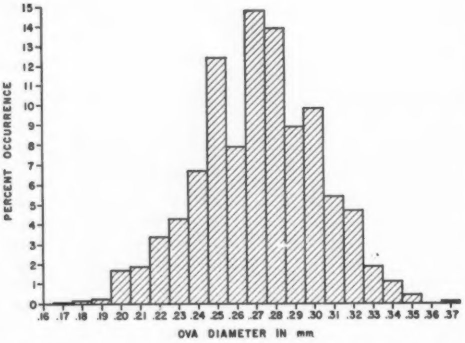


FIG. 2. Frequency distribution of ova diameters from 21 specimens of the American eel (*Anguilla rostrata*) migrating from Chesapeake Bay on November 23, 1970.

Discussion

Fecundity estimates of American eels from Chesapeake Bay are lower than those stated by Bigelow and Schroeder (1953), Vladykov (1955), or Eales (1968). This lower estimate was not caused by the destruction of eggs by the sonic cleaner because exposure time did not affect egg counts. The largest female studied was 724 mm long and weighed 755 g. This specimen has an observed fecundity of 2,561,000. The possibility that larger specimens have fecundity estimates closer to the stated values of previous authors cannot be positively excluded because the predictive value of a regression line is strictly valid only over the range of observed values. The specimens utilized in this study were representative of the size of large yellow eels in Chesapeake Bay subestuaries. The largest specimen that we have encountered during extensive trawling operations over a period of 4 yr was 812 mm.

The gonads of female silver eels migrating from Chesapeake Bay comprise a mean of 4.81% of the total weight of the animal. This value probably increases as the migratory females approach the Sargasso Sea. The maturity index was significantly correlated with the mean ova diameter in the 21 specimens used for fecundity studies ($r = 0.81$, coefficient of determination = 65.5%). This relation is obvious because as more material is incorporated into the individual eggs, thereby increasing their diameter, the gonadal weight increases thus elevating the maturity index. Wenner (1973) suggested that silver eels migrate sooner and

TABLE 1. Comparative data of gonadal conditions of female silver Atlantic eels.

Species	Location	$\bar{X}$ ova diameter (*mm*)	Extremes (*mm*)	$\bar{X}$ maturity index
A. rostrata	Newfoundland (Gray and Andrews 1970)	0.165	0.109–0.214	–
A. rostrata	SE of Cape Cod (Wenner 1973)	0.172	0.12–0.27	–
A. rostrata	E of Assateague Island (Wenner 1973)	0.356	0.25–0.45	0.0587
A. rostrata	SE of Chesapeake Bay (Wenner 1973)	0.275	0.17–0.37	–
A. rostrata	Chesapeake Bay (present study)	0.244	0.17–0.37	0.0481
A. anguilla	Danish waters (?) (Rasmussen 1951)	–	0.10–0.20	–
A. anguilla	Danish waters (?) (Bruun et al. 1949)	–	0.12–0.18	–

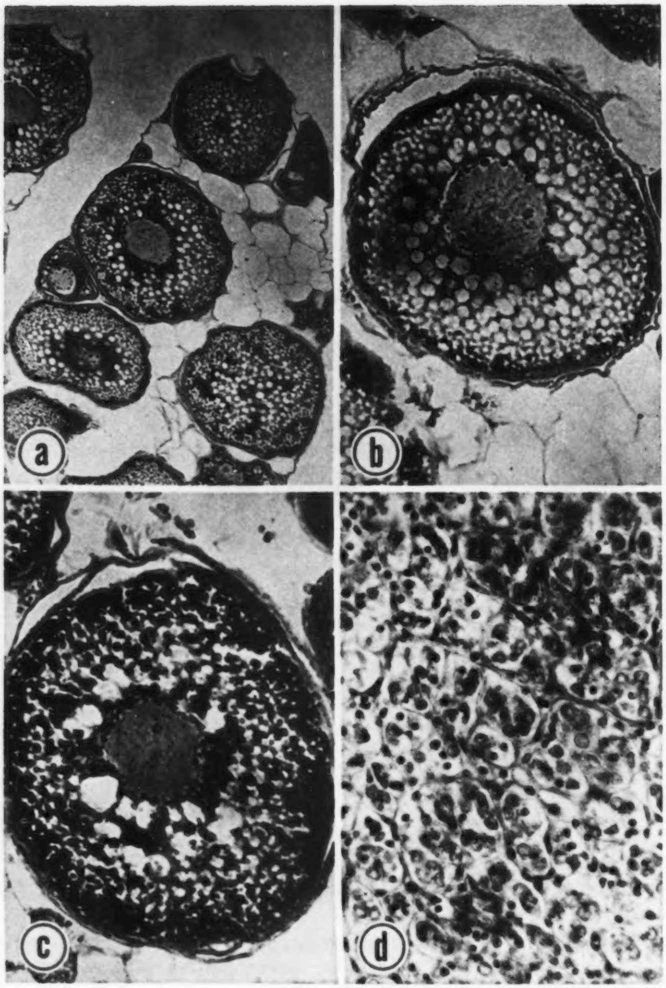


FIG. 3. Sections of gonadal tissue from migratory silver eels, fixed in Bouin's and stained with Harris-hematoxylin-eosin. a) LS ovarian tissue. Total length of specimen = 645 mm; total weight = 545 g. Horizontal diameter of oocyte in center of field = 0.24 mm; mean horizontal diameter of 10 oocytes = 0.21 mm. Note large amount of lipid tissue between developing oocytes. b) TS oocyte. Total length of specimen = 613 mm; total weight = 424 g. Horizontal diameter of oocyte = 0.22 mm; mean horizontal diameter of 10 oocytes = 0.22 mm. Note the thin layer of connective tissue surrounding the oocyte, developing vitelline membrane, yolk granules around the periphery of the cell, cytoplasmic vesicles devoid of contents which were presumably lipids lost in histological preparation, and basophilic inclusions around the inner margin of the nuclear membrane referred to by Combs (1969) in *Brevoortia patronus* oocytes as proto-vitellonucleoli. c) TS oocyte. Total length of specimen = 624 mm; total weight = 550 g. Horizontal diameter of oocyte = 0.26 mm; mean horizontal diameter of 10 oocytes = 0.27 mm. Note large lipid vesicles and more densely packed yolk granules. d) TS testis. Total length = 378 mm; total weight = 91 g. Distance across field = 0.25 mm. Note nests of secondary spermatogonia as defined by Hyder (1969).

at a more immature state in northern areas of their geographical distribution than in southern areas thus providing a mechanism whereby they may reach the spawning grounds at approximately the same time and in the same reproductive condition. To validate this hypothesis, maturity indices of specimens of different estuarine origins along a latitudinal gradient should be examined at the time of seaward migration.

The horizontal diameters of eggs treated with Gilson's solution agreed closely with those determined by sectioning tissue fixed in Bouin's (means ± 95% confidence intervals: Bouin's: 0.25 ± 0.001 mm; Gilson's: 0.24 ± 0.004 mm). These diameters are larger than those reported by Gray and Andrews (1970) for the American eels migrating from Newfoundland waters where the mean value was 0.165 (range: 0.109–0.214 mm). Rasmussen (1951) stated that the average egg diameter of migratory European eels, *A. anguilla*, is from 0.1 to 0.2 mm whereas Brunn et al. (1949) reported three untreated *A. anguilla* in an endocrinological study as having ova diameters of 0.18, 0.13, and 0.12 mm. From these data, it is concluded that migratory silver American eels leaving Chesapeake Bay are closer to sexual maturity than specimens leaving more northerly estuaries, and also more advanced than *A. anguilla* migrating from continental Europe (Table 1). This is in direct contradiction with the hypothesis (Tucker 1959) that the European eel is much more sexually advanced than the American eel at the onset of migration.

The eggs are presumed to be pelagic and about 1 mm in diameter when spawned (Bertin 1956). The presence of large cytoplasmic vesicles devoid of contents in histological preparations indicates the large amount of lipid materials in the egg. Combs (1969) described the peripheral displacement of the nucleus and the formation of a centrally located oil globule in the pelagic eggs of *Brevoortia patronus*. This aggregation of oil droplets in the eggs of *A. rostrata* was not demonstrated in the specimens observed but cannot be ruled out. A similar flotation mechanism may be employed as the spawning grounds are approached.

Histological sections showed that all male specimens had small, rather undeveloped testes markedly immature by comparison to a male *A. anguilla* pictured in Bertin (1956) and those brought to a spawning condition by Boetius and Boetius (1967). The organs of Syrski were small and vascularized and it was evident that much development must take place before spawning. Excellent gross morphological descriptions of the ovaries and testis of the European eel may be found in the descriptions of Goode (1881).

Acknowledgments

We thank Dr F. O. Perkins and his competent technical staff, especially Mrs P. Berry, for providing histological preparations. Dr M. Chittenden contributed statistical advice and Dr B. Collette of the National Marine Fisheries Service Systematic Laboratory, Washington, D.C. reviewed the manuscript.

BAGENAL, T. B. 1967. A short review of fish fecundity, p. 89–111. *In* S. D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell Scientific Publ., Oxford.

BERTIN, L. 1956. Eels: a biological study. Cleaver-Hume Press, Ltd., London. 192 p.

BIGELOW, H. B., AND W. C. SCHROEDER. 1953. Fishes of the Gulf of Maine. U.S. Fish Wildl. Serv. Fish. Bull. 53: 150–160.

BOETIUS, I., AND J. BOETIUS. 1967. Studies in the European eel, *Anguilla anguilla* (L.). Experimental induction of the male sexual cycle, its relation to temperature and other factors. Medd. Dan. Fisk. Havunders 4: 339–405.

BRUUN, A. F., A. M. HEMMINGSEN, AND E. MØLLER-CHRISTENSEN. 1949. Attempts to induce experimentally maturation of the gonads of the European eel, *Anguilla anguilla* L. Acta Endocrinol. 2: 212–226.

COMBS, R. M. 1969. Embryogenesis, histology and organology of the ovary of *Brevoortia patronus*. Gulf. Res. Rep. 2: 333–434.

EALES, J. G. 1968. The eel fisheries of eastern Canada. Bull. Fish. Res. Board Can. 166: 79 p.

EGE, V. 1939. A revision of the genus *Anguilla* (Shaw), a systematic, phylogenetic and geographical study. Dana Rep. Carlsberg Found. 16: 1–256.

GOODE, G. B. 1881. Notes on the life history of the eel, chiefly derived from a study of recent European authorities. Bull. U.S. Fish Comm. 1: 71–130.

GRAY, R. W., AND C. W. ANDREWS. 1970. Sex ratio of the American eel, *Anguilla rostrata* (Lesueur), in Newfoundland waters. Can. J. Zool. 48: 483–487.

GUENTHER, W. C. 1964. Analysis of variance. Prentice-Hall Inc., Englewood Cliffs, N.J. 199 p.

HYDER, M. 1969. Histological studies on the testis of *Tilapia leucosticta* and other species of the genus *Tilapia* (Pices: Teleostei). Trans. Am. Microsc. Soc. 88: 211–231.

RASMUSSEN, C. J. 1951. Two Danish finds of female eel (*Anguilla anguilla*) in spawning or partial spawning dress. Rep. Dan. Biol. Sta. 53: 36–39.

SOKAL, R. R., AND F. J. ROHLF. 1969. Biometry. W. H. Freeman and Co., San Francisco, Calif. 766 p.

TUCKER, D. W. 1959. A new solution to the Atlantic eel problem. Nature 183: 496–501.

VLADYKOV, V. D. 1955. Eels. Poissons du Québec. Dep. of Fish., Québec. 12 p.

WENNER, C. A. 1973. Occurrence of American eels, *Anguilla rostrata*, in waters overlying the eastern North American continental shelf. J. Fish. Res. Board Can. 30: 1752–1755.

NOTES

Effects of Reduced Oxygen on the Embryos and Larvae of Lake Trout (*Salvelinus namaycush*) and Largemouth Bass (*Micropterus salmoides*)

ANTHONY R. CARLSON AND RICHARD E. SIEFERT

U.S. Environmental Protection Agency
National Water Quality Laboratory, Duluth, Minn. 55804, USA

CARLSON A. R., AND R. E. SIEFERT. 1974. Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). J. Fish. Res. Board Can. 31: 1393–1396.

At 7 and 10 C lake trout (*Salvelinus namaycush*) development from fertilization through first feeding was inhibited at all reduced oxygen saturations tested (50% and lower). Survival was adversely affected at all reduced concentrations although only slightly at 50% saturation at 7 C.

At 20 and 23 C oxygen concentrations as low as 35% saturation were adequate for largemouth bass (*Micropterus salmoides*) survival. However, even at the highest reduced oxygen saturation (70%) growth was reduced, and at saturations of 50% and below hatching was premature and first feeding was delayed.

CARLSON A. R., AND R. E. SIEFERT. 1974. Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). J. Fish. Res. Board Can. 31: 1393–1396.

At 7 et 10 C, le développement du touladi (*Salvelinus namaycush*), de la fécondation au moment où il se nourrit pour la première fois, est inhibé à toutes les saturations réduites d'oxygène essayées (50% et moins). Toutes les concentrations réduites ont une influence défavorable sur la survie, bien qu'un peu seulement à 50% de saturation et à 7 C.

A 20 et 23 C, des teneurs en oxygène aussi basses que 35% de saturation sont adéquates pour la survie de l'achigan à grande bouche. Cependant, même à la saturation réduite la plus élevée en oxygène (70%), la croissance est ralentie, et, à des saturations de 50% et moins, l'éclosion est prématurée et la première prise de nourriture retardée.

Received November 23, 1973
Accepted April 23, 1974

Reçu le 23 novembre, 1973
Accepté le le 23 avril, 1974

THE high discharge rates of oxygen-demanding effluents from industrial, agricultural, and domestic sources necessitate the study of the effects of lowered dissolved oxygen on important fish species. Knowledge of these effects on the more susceptible embryonic and larval stages is essential to providing meaningful water quality criteria. This report is part of a program at the National Water Quality Laboratory to determine the environmental requirements of important freshwater aquatic life.

The objective of this study was to determine the effects of reduced dissolved oxygen concentrations on development and survival of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*) embryos and larvae reared through the first-feeding stage. The effects of reduced oxygen on the development rate of lake trout embryos have been studied by Garside (1959), but included only limited information on embryo survival. Dudley (1969) has described the effects of low oxygen on the survival and hatching of largemouth bass embryos. We have found no literature pertaining to larval development and survival at reduced oxygen concentrations for either species.

Printed in Canada (J3231)
Imprimé au Canada (J3231)

Materials and methods — The apparatus used to deliver the desired dissolved oxygen concentrations and the experimental chambers were similar to those described by Siefert et al. (1973), with the following exceptions: mixed gas atmospheres were not used; #40 stainless steel mesh replaced the nylon mesh in the duplicate experimental chambers; and inlet compartments were shortened by 4 cm, so that the experimental chambers were 26 cm long, with a capacity of 3.4 liters.

The dissolved oxygen concentrations were lowered in a vacuum degassing system similar to that described by Mount (1964) and were measured daily by the modified Winkler method. Measurements were alternated between duplicate experimental chambers. Nominal dissolved oxygen values are used for simplicity in the presentation and discussion of the results.

Untreated Lake Superior water was filtered to remove particles larger than 10 μ. Chemical characteristics of the water, measured weekly during the test periods according to methods described by the American Public Health Association (1965), were (in mg/liter): total alkalinity, 41–42; total hardness, 43–44; and acidity, 2.97–3.96. The pH in all experimental chambers ranged between 7.6 and 7.8. Water flow to each chamber was 60 ml/min (velocities past the two different size embryos: lake trout, 7.2 cm/min; largemouth bass, 3.0 cm/min). Lighting at 10–15 ft-c was provided by Duro-test Optima[1] lamps. Test temperatures were 7 and 10 C for lake trout and 20 and 23 C for largemouth bass, and were within the optimium range and near the higher limit for incubation of each species (McAfee 1966; Emig 1966).

Lake trout brood stock was trapnetted from Lake Superior near Marquette, Michigan. The fish were stripped on October 31, 1972, and the eggs were fertilized by the dry method (Sorenson et al. 1966). Water temperature was 6 C at stripping. The embryos were placed in a styrofoam container, covered, and immediately transferred to the laboratory. Six hours later and at 7 C, 95 embryos were placed randomly into each experimental chamber. Development and survival testing was done at dissolved oxygen concentrations of approximately 15, 20, 35, 50, and 100% saturation at 7 C and 20, 30, 40, 50, and 100% saturation at 10 C. Each experimental chamber temperature was measured daily and they averaged 6.9 (6.6–7.6) and 10 (9.4–10.2) C with standard deviations of ± 0.1 C. The chambers were darkened until hatching was complete; thereafter a photoperiod of 11 h, representing natural seasonal conditions, was used.

Largemouth bass embryos were obtained from fish that had spawned in the laboratory at 21 C; methods used are described by Carlson and Hale (1972). One hundred and ten embryos from eggs that were fertilized during the previous 16 h were placed randomly into each experimental chamber on April 27, 1973. Development and survival testing was done at dissolved oxygen concentrations of approximately 20, 35, 50, 70, and 100% saturation. Experimental chamber temperatures averaged 20.1 (19.9–20.2) and 22.9 (22.8–23.3) C with standard deviations of ± 0.1 and ± 0.2, respectively. A photoperiod of 15 h was used.

The time of first feeding of lake trout was determined by offering live brine shrimp (*Artemia salina*) to the larvae and noting the orange color of the shrimp in the digestive tract. Thereafter the larvae were fed an excess of Oregon Moist trout food 3 times each day. Largemouth bass were fed laboratory-cultured plankton 3 times a day. One-half hour after each plankton feeding they were fed an excess of live brine shrimp. First feeding was determined as described for lake trout. Fecal matter and excess food were siphoned from the chambers approximately 1 h after feeding. The experiments were ended when all larvae had started to feed.

Results and discussion — Lake trout — At 7 C lake trout at 100% saturation (controls) began hatching 70 days after fertilization, completed 90%

TABLE 1. Times to hatching and feeding, and total lengths and survival of lake trout (*Salvelinus namaycush*) at the oxygen concentrations and temperatures indicated, and test durations of 131 days at 7 C and 108 days at 10 C. Values are for duplicate experimental chambers; 95 embryos initially in each chamber.

Oxygen concn								
% saturation[a]		mg/liter		Time in days to:				
Nominal	Actual (mean)	Mean ± SD	Range	Initial hatch	90% hatch	Initial feeding	Total length at test end (*mm*)	Survival at test end (%)
				7 C				
100	95	11.4±0.4	10.5–12.0	70	81	101	26.5(24.0–29.5)[b]	75.8(75.8)[b]
50	50	6.0±0.2	5.3–6.5	72	84	108	24.8(22.8–26.6)	71.1(69.5–72.7)
35	35.8	4.3±0.2	3.7–4.8	72	87	113	23.8(22.1–25.0)	61.6(60.0–63.2)
20	20.0	2.4±0.3	1.9–3.2	75	88	130	– –	0
15	14.1	1.7±0.3	1.4–2.7	–	–	–	– –	0
				10 C				
100	93.7	10.5±0.4	9.5–11.2	49	56	60	26.9(21.8–30.3)	58.9(52.6–65.3)
50	50.0	5.6±0.2	5.2–6.1	51	58	76	23.6(21.8–25.5)	23.1(17.9–28.4)
40	40.2	4.5±0.2	4.1–5.0	52	58	99	23.0 –	0.5(0.0–1.0)
30	30.4	3.4±0.2	3.0–4.2	52	54	–	– –	0
20	20.5	2.3±0.2	1.8–2.7	–	–	–	– –	0

[a] In relation to atmosphere air.
[b] Mean and range.

---

[1] The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

hatch by day 81, and began feeding on day 101 (Table 1). At the end of the test, average fish length was 26.5 mm and survival was 75.8%. Effects on development and survival were evident at all reduced oxygen concentrations and became more pronounced as the concentration decreased. Initial hatch was delayed by 2–5 days, first feeding was delayed by 7–29 days, and fish length was reduced by 1.7–2.7 mm. Survival was slightly reduced at 50 and 35% saturation; at 20% a few larvae survived through hatching, but died within 24 h after first feeding. No hatching took place at 15% saturation.

At 10 C lake trout developed faster than at 7 C, but did not survive as well at comparable oxygen concentrations (Table 1). Control fish began hatching after 49 days of incubation, completed 90% hatch 7 days later, and began feeding on the 60th day. At the end of the test the average length was 26.9 mm and survival 58.9%. Although there was little difference in time of initial hatch at 10 C between the control fish and those at the reduced oxygen concentrations, mean larval growth at 50% saturation was 3.3 mm less and survival 61% less than the controls. Most larvae at 40% saturation died before feeding began and no hatching occurred at 20% saturation.

Judging by the survival rate, 7 C and dissolved oxygen near air-saturation were the most favorable conditions tested in the study. Although 50% saturation was less favorable, it does not appear to be too low for the species at this temperature. We consider 35% saturation too low, not only because the survival rate is lower (19% lower than the control), but also because it borders on a concentration shown to be lethal.

Apparently 10 C is too warm, as even at near air-saturation of dissolved oxygen survival was no better than at 35% saturation at 7 C. In agreement are the findings of Garside (1959), who tested lake trout embryos at various temperatures and at oxygen concentrations of 2.5, 3.5, and 4.5 mg/liter; all embryos died before or within a few hours of hatching at 10 C but embryos at lower temperatures and the same concentrations survived.

In a study with methods and endpoints similar to those of the present one, brook trout (*Salvelinus fontinalis*) showed slight delays in development at 50% saturation, but marked effects on development and poor survival occurred only at 20% saturation and below (Siefert and Spoor 1974). In contrast, lake trout survival at concentrations of 35% saturation at 7 C and 50% saturation at 10 C was relatively poor. Garside (1959) concluded that at oxygen saturations below 40%, and at 3 C or less, lake trout embryos may be subjected to stress. This lower tolerance of early stage lake trout to reduced oxygen concentrations may well be attributed to

TABLE 2. Times to hatching and feeding, and total lengths and survival at 20 days after test initiation, for largemouth bass (*Micropterus salmoides*) at the oxygen concentrations and temperatures indicated. Values are for duplicate experimental chambers; 110 embryos initially in each chamber.

Oxygen concn								
% saturation[a]		mg/liter		Time in hours to:				
Nominal	Actual (mean)	Mean ± SD	Range	Initial hatch	90% hatch	Initial feeding	Total length at 20 days (*mm*)	Survival at 20 days (%)
				20 C				
100	94.5	8.6±0.2	8.1–9.0	50[b]	64	193	11.6(9.4–13.0)[c]	89.1(88.2–90.0)[c]
70	69.2	6.3±0.2	5.8–6.7	56	64	195	10.7(9.0–12.1)	88.2(87.3–89.1)
50	49.4	4.5±0.2	4.1–5.1	49	59	204	10.0(8.1–11.6)	88.2(87.3–89.1)
35	34.1	3.1±0.2	2.8–3.5	51	58	216[d]	9.8(9.1–11.0)	75.4(67.3–83.6)
20	18.7	1.7±0.1	1.4–1.7	47	57	220	9.0(9.0)	0.4(0.0–0.9)
				23 C				
100	97.6	8.3±0.2	7.8–8.5	–[e]	–[e]	124	14.6(11.2–16.7)	74.5(72.7–76.4)
70	70.6	6.0±0.2	5.6–6.3	–	–	124	13.8(10.9–16.0)	89.1(89.1)
50	49.4	4.2±0.2	3.9–4.8	–	–	129	13.4(9.3–15.5)	61.8(57.3–66.4)
35	35.3	3.0±0.1	2.8–3.3	–	–	170	11.8(9.0–14.4)	77.2(73.6–80.9)
20	20.0	1.7±0.1	1.6–1.9	–	–	–	– –	0

[a] In relation to atmosphere air.

[b] One hatch until hour 59.

[c] Mean and range.

[d] Feeding observed at this time, but probably started sometime in the previous 12 h.

[e] Hatching was not observed, but was completed in all chambers sometime between hour 31 and 47.

the differences in natural spawning habitats between the two species. Brook trout spawn in streams and lakes, burying the embryos in redds where the oxygen concentrations may be low, whereas lake trout usually spawn over rubble in well-oxygenated lakes.

The differences between the oxygen requirements of these related species illustrate that a species' entire life history should be known if the results of laboratory experiments are to be applied with confidence to natural populations. So far as survival and development under the conditions of the present experiment are concerned, near air-saturation is better than 50 or 35%, and 7 C is better than 10 C, but these may not be the important results in the natural state. Providing there is enough oxygen that some can survive, delayed hatching or feeding might be more important to the species, helping to adjust to weather conditions, food organism availability and pulses, or predator–prey relationships. Even increased mortality might allow increased production by reducing intraspecific competition. Assuming, however, that poor larval survival and development indicate poor conditions for lake trout, we can conclude that the oxygen concentrations should be no less than 50% saturation at 7 C.

Largemouth bass — At 20 C first hatch of largemouth bass at 100% saturation (control) occurred after 50 h of exposure (Table 2). This initial hatch was probably premature as the next control embryo hatched 9 h later. Ninety percent hatch was completed after 64 h, and feeding began after 193 h. Average fish length was 11.6 mm and survival was 89.1% at the end of the test (20 days). At 70% saturation the time for completion of hatch was the same as in the controls, and time of first feeding was similar to that in the controls. Hatching was completed slightly earlier at the remaining reduced oxygen concentrations. First feeding was delayed 11, 23, and 27 h at 50, 35, and 20% saturation, respectively. Growth in length was retarded at all reduced saturations with average lengths 0.9–2.6 mm less than the controls. Survival at 50 and 70% saturation was similar to the controls, at 35% it was 13.7% less, and at 20% only one larva lived.

At 23 C development of largemouth bass was faster than at 20 C, and survival was similar but more variable (Table 2). First feeding was delayed at concentrations of 50% or less, and growth was reduced with each successive lowering of the oxygen concentration.

As with lake trout, the ecological significance of these reduced survival rates and delays in development at the intermediate oxygen concentrations is obscure. At temperatures of 20 and 23 C, dissolved oxygen concentrations down to 35% saturation appear adequate for good survival of largemouth bass embryos and larvae. Dudley (1969) found that the survival of largemouth bass embryos decreased sharply at 2.0, 2.0, and 2.8 mg/liter at 15, 20, and 25 C, respectively (about 20–35% saturation). Field studies in Lake George, Minnesota, showed no apparent relationship between embryo survival and oxygen concentrations ranging from 5.8 to 12.8 mg/liter (Kramer and Smith 1962). However, in our study even at the highest reduced oxygen concentration, 70% saturation, growth was lessened, and at 50% saturation first feeding was delayed.

Acknowledgments — We thank L. J. Herman for technical assistance during the studies, Dr W. A. Spoor for technical and editorial guidance, and the Michigan Department of Natural Resources for the lake trout embryos.

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1965. Standard methods for the examination of water and wastewater. 12th ed. New York, N.Y. 769 p.

Carlson, A. R., and J. G. Hale. 1972. Successful spawning of largemouth bass (*Micropterus salmoides* (Lacépède)) under laboratory conditions. Trans. Am. Fish. Soc. 101: 539–542.

Dudley, G. D. 1969. Survival of largemouth bass embryos at low dissolved oxygen concentrations. M.Sc. Thesis. Cornell Univ. Ithaca, N.Y. 61 p.

Emig, J. W. 1966. Largemouth bass, p. 332–353. *In* A. Calhoun [ed.] Inland fisheries management. Calif. Dep. Fish Game Fish Bull.

Garside, E. T. 1959. Some effects of oxygen in relation to temperatures on the development of lake trout embryos. Can. J. Zool. 37: 689–698.

Kramer, R. H., and L. L. Smith. 1962. Formation of year classes in largemouth bass. Trans. Am. Fish. Soc. 91: 29–41.

McAfee, W. R. 1966. Lake trout, p. 260–271. *In* A. Calhoun [ed.] Inland fisheries management. Calif. Dep. Fish Game Fish Bull.

Mount, D. I. 1964. Additional information on a system for controlling the dissolved oxygen content of water. Trans. Am. Fish. Soc. 93: 100–103.

Siefert, R. E., and W. A. Spoor. 1974. Effects of reduced oxygen on embryos and larvae of the white sucker, coho salmon, brook trout, and walleye. *In* J. H. S. Blaxter [ed.] Proc. Int. Symp. on the Early Life History of Fish, Oban, Scotland, 1973. Springer-Verlag, Heidelberg, W. Germany. (In press)

Siefert, R. E., W. A. Spoor, and R. F. Syrett. 1973. Effects of reduced oxygen concentrations on northern pike (*Esox lucius*) embryos and larvae. J. Fish. Res. Board Can. 30: 849–852.

Sorenson, L., K. Buss, and A. R. Bradford. 1966. The artificial propagation of esocid fishes in Pennsylvania. Prog. Fish-Cult. 28: 133–141.

Liver Damage in Rainbow Trout (*Salmo gairdneri*) Fed Cyclopropenoid Fatty Acids[1]

Y. MALEVSKI, J. H. WALES, AND M. W. MONTGOMERY

Department of Food Science and Technology
Oregon State University, Corvallis, Oreg. 97331, USA

MALEVSKI, Y., J. H. WALES, AND M. W. MONTGOMERY. 1974. Liver damage in rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids. J. Fish. Res. Board Can. 31: 1397–1400.

Histological changes in livers of rainbow trout (*Salmo gairdneri*) repeatedly fed diets containing cyclopropenoid fatty acids (CPFA) revealed that these fish were unable to develop resistance to liver damage caused by CPFA. One group of trout was fed 150 ppm CPFA in the basal diet for 49 days followed by the basal diet for the remainder of the experimental period. Another group of trout was fed 150 ppm CPFA for 49 days, basal diet for 65 days, and 150 ppm CPFA for 45 days. At this time this group was divided into two subgroups; one received basal diet for 15 days, the other for 30 days, after which both subgroups were placed on a diet containing 360 ppm CPFA for 36 days. Analysis of the livers for histological changes showed that the parenchymal cells underwent cyclic changes of injury and recovery each time the trout were fed CPFA. Complete recovery of parenchymal cells occurred after the fish were removed from the diets containing CPFA for 30 days. Bile duct and blood vessel hyperplasia was observed for as long as 7 mo after CPFA was eliminated from the trout diet.

MALEVSKI, Y., J. H. WALES, AND M. W. MONTGOMERY. 1974. Liver damage in rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids. J. Fish. Res. Board Can. 31: 1397–1400.

Les changements histologiques qui se produisent dans le foie de truites arc-en-ciel (*Salmo gairdneri*) nourries à plusieurs reprises de régimes contenant des acides gras cyclopropénoïdes (CPFA) indiquent que ces poissons ne peuvent développer de résistance aux dommages causés au foie par les CPFA. On a donné à un groupe de truites 150 ppm de CPFA dans le régime de base pendant 49 jours, et le régime de base seulement pour le reste de l'expérience. On a donné à un autre groupe de truites 150 ppm de CPFA pendant 49 jours, le régime de base pendant 65 jours et 150 ppm de CPFA pendant 45 jours. On a alors divisé ce groupe en deux sous-groupes; un groupe a reçu le régime de base pendant 15 jours, l'autre pendant 30 jours, après quoi les deux sous-groupes furent soumis à un régime contenant 360 ppm de CPFA pendant 36 jours. L'examen histologiques des foies démontre que les cellules du parenchyme subissent des changements cycliques, dommage et récupération, chaque fois que les truites reçoivent des CPFA. Il y a récupération complète des cellules du parenchyme une fois les poissons éloignés pendant 30 jours des régimes contenant des CPFA. Nous avons observé une hyperplasie du conduit biliaire et des vaisseaux sanguins aussi longtemps que 7 mois après élimination des CPFA dans le régime des truites.

Received May 7, 1973
Accepted April 18, 1974

Reçu le 7 mai, 1973
Accepté le 18 avril, 1973

WOLF and Jackson (1963) and Sinnhuber et al. (1965) traced an epizootic of liver cancer in hatchery-reared rainbow trout (*Salmo gairdneri*) to aflatoxin contaminated cottonseed meal in the diet. In a search for other carcinogens in cottonseed meal, Sinnhuber et al. (1966, 1968) found that cyclopropenoid fatty acids (CPFA), natural components of cottonseed lipids, increased the incidence of hepatomas in aflatoxin B_1-fed rainbow trout.

Although CPFA are not carcinogenic to rainbow trout, these ring-containing fatty acids produce certain biochemical and morphological changes in livers of trout (Lee et al. 1968; Roehm 1968; Taylor et al. 1973). Histological examination of livers from CPFA-fed trout revealed that regeneration occurred after 6–12 mo of CPFA feeding (Wales 1967). This suggests that trout livers may

---

[1] Technical Paper No. 3589, Oregon Agricultural Experiment Station.

Printed in Canada (J3023)
Imprimé au Canada (J3023)

develop resistance to histological damage caused by CPFA.

To study this possible development of resistance to CPFA, the histological changes that occur in rainbow trout livers after repeated feeding of CPFA were determined. If the livers developed a resistance to CPFA damage, histological changes would be decreased or eliminated upon further exposure to CPFA.

Materials and methods — Five-month-old (3–4 g) rainbow trout (*Salmo gairdneri*) of the Mt. Shasta strain used in this study, were hatched and reared in our laboratory as described by Lee et al. (1967). Two groups of 95 fish each were placed in perforated plastic buckets which were hung on the inside of a 757-liter (200-gal) fiberglass tank. Water at a flow rate of 19 liters (5 gal)/min and 12.5 C was allowed to flow through the tank. When the trout attained an average weight of 10 g, each group was transferred to separate fiberglass tanks. They were first fed the basal diet (Table 1) and this was followed by the basal diet with addition of CPFA derived from *Sterculia foetida* oil.

The first group of trout was fed the 150 ppm CPFA diet for 49 days followed by the basal diet for the remainder of the experimental period (176 days). The second group was fed 150 ppm CPFA diet for 49 days, basal diet for 65 days, and 150 ppm CPFA diet for 45 days. Then this group was divided into two subgroups, one was fed the basal diet for 15 days and the other was fed the basal diet for 30 days. Then each subgroup received a diet containing 360 ppm CPFA for 36 days. Previous observations had revealed that no histological changes occur in the livers of trout on the basal diet.

TABLE 1. Composition of basal diet.

Ingredient	%
Casein (NBC vitamin-free)	49.5
Dextrose[a]	15.6
Herring oil	10.0
Gelatin	8.7
Alpha-cellulose (Alphacel-NBC)	7.7
Mineral mix[b]	4.0
Vitamin mix[c]	2.0
Carboxy-methyl cellulose[d]	1.3
70% choline chloride	1.0
Vitamin E (supplies 660 IU/kg)	0.2

[a] Cerelose, CPC International, Inc., Englewood Cliffs, N.J. 07632, USA.

[b] Bernhart-Tomerelli Salt Mix, J. Nutr. 89:495, 1966, modified by addition of NaF and $CoCl_2$ at 0.002 and 0.020%, respectively.

[c] As described by Lee et al. (1967), with the exception of cobalamine being fed at the level of 0.053 mg/kg instead of 0.159.

[d] Hercules Powder Co., 120 Montgomery St., San Francisco, Calif.

For each sampling period five fish were randomly sampled, their livers excised and immediately fixed in Bouin's fluid. They were later imbedded in paraffin and sectioned. After staining with hematoxylin and eosin, the liver sections were examined with a light microscope.

Results — Microscopic degenerative liver changes were detected in trout fed 150 ppm CPFA as early as 3 days on the diet. At this time the main changes consisted of vascular fibrosis (Plate I, 2 and 3; Plate II, 8). After 8 days of CPFA feeding parenchymal cell enlargement was evident and the nuclei were enlarged and irregularly shaped. (Plate I, 4; Plate II, 5). Most of the parenchymal cells contained "fibers" and sphaerical vacuoles which originally contained lipid (Plate II, 7).

After 16 days of CPFA feeding the vascular fibrosis had increased significantly and the parenchymal cells were much enlarged with vacuoles (Plate II, 7). Cell cords, normally present in control fish, were distorted (Plate I, 2). Hyperplasia of both the epithelium and the connective tissue of bile ducts and blood vessels was evident (Plate I, 2 and 3).

After 28 days of CPFA feeding the vascular fibrosis and the hyperplasia of the bile ducts and blood vessels were similar to that seen after 16 days, but the parenchymal cells were becoming increasingly vacuolated. Some regeneration of parenchymal cells was evident (Plate II, 8).

After 45 days of CPFA feeding, vascular fibrosis was less evident, many of the parenchymal cells were vacuolated, some regeneration was observed and the hyperplasia of the bile ducts and blood vessels was as severe as after 28 days.

Just after the fish were put on CPFA for the second time an examination of the livers revealed that the parenchymal cells were fully regenerated. No fibers were present and all the nuclei were normal in shape and size. However, there was residual hyperplasia of the bile duct and blood vessel walls.

In the second period of CPFA feeding, essentially the same type of degenerative and regenerative changes occurred as were described above. After 6 days of CPFA feeding, the parenchymal cells had become enlarged and full of fibers. The vacuolation was quite low, and the nuclei were picnotic. Bile ducts and blood vessels were hyperplastic. After 17, 28, and 45 days the same types of degenerative changes had occurred with two exceptions. The parenchymal cells became highly vacuolated and many young parenchymal cells were seen. The

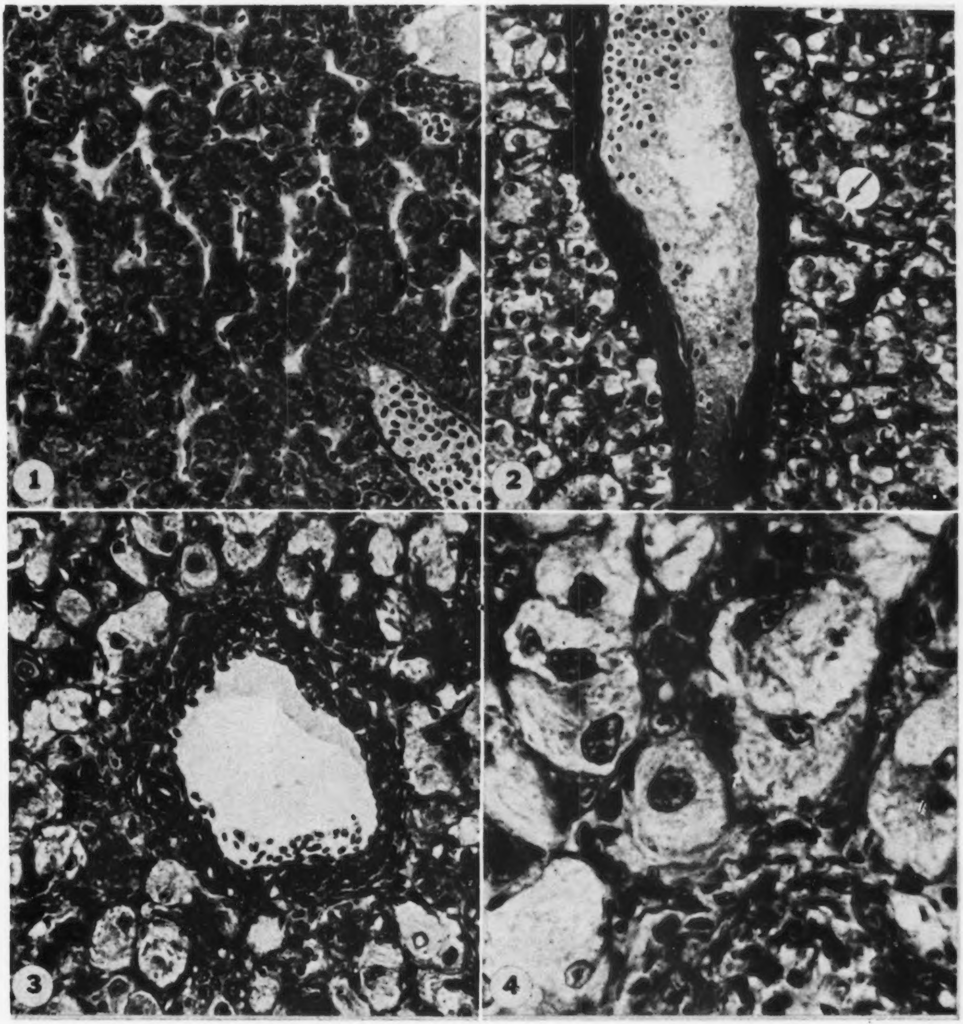


PLATE I. Parenchymal cells of liver of rainbow trout (*Salmo gairdneri*); hematoxylin and eosin stain. 1, Trout fed basal diet without CPFA. Cords two cells wide and blood vessels thin. 128 ×. 2, Trout fed 150 ppm CPFA for 16 days. Arterial walls thickened considerably and many parenchymal cells (arrow) have condensed into spherical masses of degenerated cytoplasm. 128 ×. 3, Trout fed 150 ppm CPFA for 16 days. A large proportion of the degenerating parenchymal cells have bizarre nuclei and are filled with fat globules and fiber-like strands. Scattered among the degenerating cells are colonies of small, hyperchromatic, regenerating cells. The artery in the center of the photo has developed the typical, thickened wall. 128 ×. 4, Detail of (3) showing the bizarre nuclei, often with two or more nucleoli 320 ×.

Malevski et al. — J. Fish. Res. Board Can.

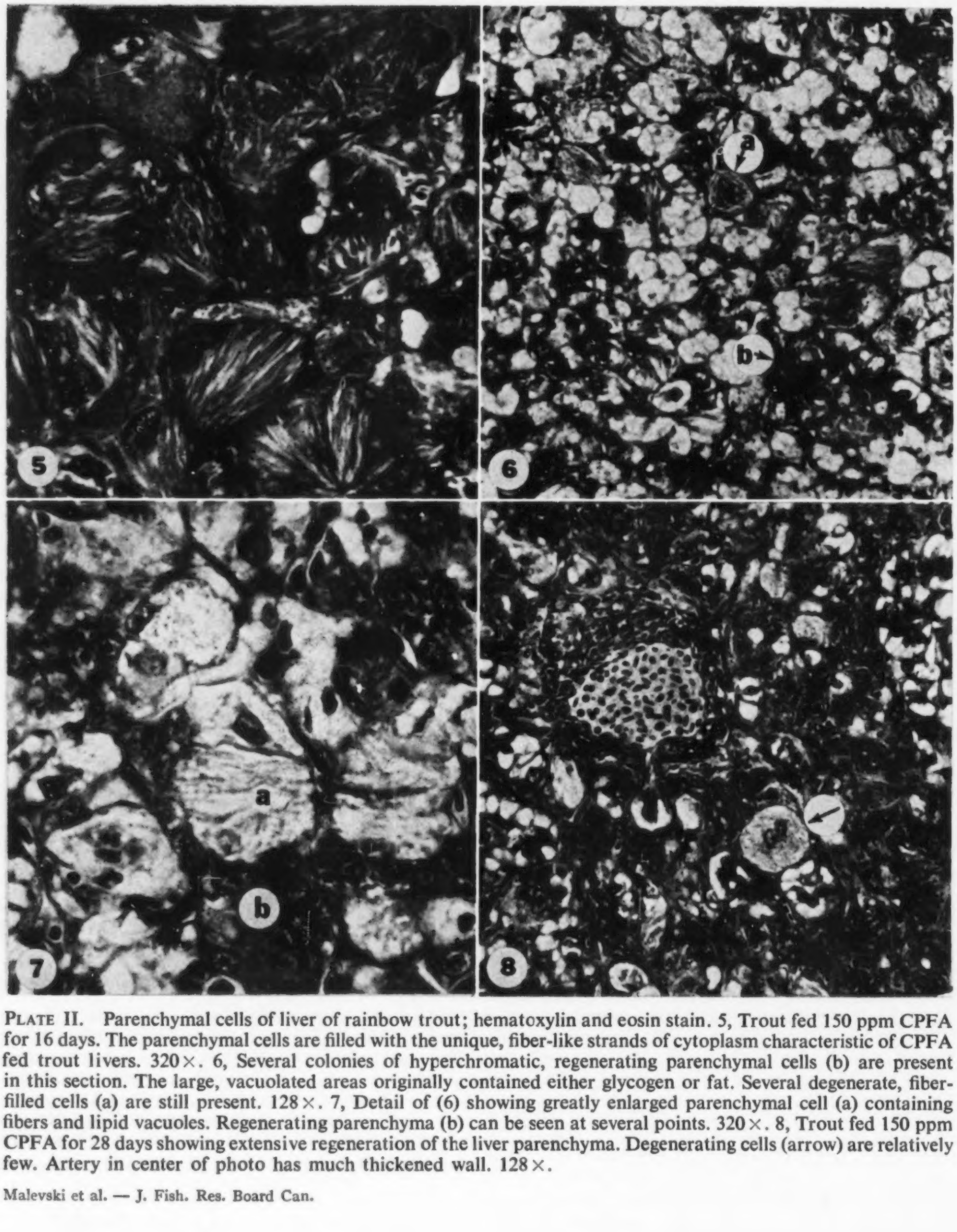


PLATE II. Parenchymal cells of liver of rainbow trout; hematoxylin and eosin stain. 5, Trout fed 150 ppm CPFA for 16 days. The parenchymal cells are filled with the unique, fiber-like strands of cytoplasm characteristic of CPFA fed trout livers. 320×. 6, Several colonies of hyperchromatic, regenerating parenchymal cells (b) are present in this section. The large, vacuolated areas originally contained either glycogen or fat. Several degenerate, fiber-filled cells (a) are still present. 128×. 7, Detail of (6) showing greatly enlarged parenchymal cell (a) containing fibers and lipid vacuoles. Regenerating parenchyma (b) can be seen at several points. 320×. 8, Trout fed 150 ppm CPFA for 28 days showing extensive regeneration of the liver parenchyma. Degenerating cells (arrow) are relatively few. Artery in center of photo has much thickened wall. 128×.

Malevski et al. — J. Fish. Res. Board Can.

regeneration was never greater than 50% of the total liver volume.

After 15 days on the basal diet and just before the start of the third period of CPFA feeding, histological examination revealed that the livers had not completed regeneration. Some cells were filled with fibers and some distorted nuclei were present. Bile duct and blood vessel hyperplasia was evident. However, after 30 days on the basal diet and just before the initiation of the third period of CPFA feeding all parenchymal cells were fully recovered but hyperplasia was still present in the bile ducts and blood vessels.

Feeding CPFA in the third period caused similar changes as described above. However, regeneration occurred somewhat more slowly, probably due to the higher level of CPFA used during this period.

The first group of trout was fed 150 ppm CPFA for 49 days and the basal diet thereafter. While being fed CPFA, the livers showed typical changes described above. When put on the basal diet, the parenchymal cells recovered within 30 days, but bile duct and blood vessel hyperplasia continued for the entire observation period.

Discussion — The cyclic changes of liver degeneration and regeneration caused by CPFA feeding in rainbow trout are not unique to these substances. Wunder (1971) found that aflatoxin B_1 caused a similar pattern of liver pathology.

Based on results presented above and in the literature, some general correlations between physiological and histological liver changes can be drawn. Roehm et al. (1970) reported that with trout fed 50 ppm CPFA, food intake dropped and the rate of body weight gain decreased for 3 mo. This was followed by a period of normal food consumption and body weight gain. In another study, trout liver protein synthesis was reduced during the first 2 wk of feeding 360 ppm CPFA and then returned to normal (Malevski et al. 1973). When trout fingerlings were fed 50 ppm CPFA for 12 mo, the livers showed increased weight over the control group for the entire experimental period (Roehm 1968).

Histologically, some of the changes observed in this study had only a limited duration and others lasted for longer periods. Parenchymal cell hyperplasia lasted as long as CPFA was fed. Once CPFA were removed from the diet the parenchymal cells quickly regenerated, and within 30 days no damaged cells were observed. However, bile duct and blood vessel hyperplasia lasted for the entire observation period of 7 mo after discontinuance of CPFA feeding.

In the three CPFA feeding periods, fully recovered parenchymal cells were not observed and regeneration was never greater than 50% of the total liver volume. This suggests that parenchymal cell degeneration and recovery were at equilibrium while CPFA was in the diet. As each population of cells in the different feeding periods was equally susceptible to CPFA damage and the parenchymal cells did not fully recover in this study or in another experiment where CPFA were fed for 8 mo (B. V. Struthers personal communication), it appears that the rainbow trout used in this study are incapable of metabolizing CPFA or at least have difficulty doing so. Further it appears that regenerated cell populations are also incapable of such metabolism.

Acknowledgments — This investigation was supported in part by USPHS grant ES00550. Also, we would like to express our gratitude for the technical assistance of Mrs L. J. Hunter, Mr T. Will, and Mr R. A. Foster.

LEE, D. J., J. N. ROEHM, T. C. YU, AND R. O. SINNHUBER. 1967. Effect of w3 fatty acids on the growth rate of rainbow trout, *Salmo gairdnerii*. J. Nutr. 92: 93–98.

LEE, D. J., J. H. WALES, J. L. AYRES, AND R. O. SINNHUBER. 1968. Synergism between cyclopropenoid fatty acids and chemical carcinogens in rainbow trout (*Salmo gairdneri*). Cancer Res. 28: 2312–2318.

MALEVSKI, Y., M. W. MONTGOMERY, AND R. O. SINNHUBER. 1973. Liver composition and protein synthesis in rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids. Fed. Proc. 32: 277.

ROEHM, J. N. 1968. Changes in the lipid composition of rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids. Ph.D. Thesis. Oregon State University, Corvallis, Oreg. 82 p.

ROEHM, J. N., D. J. LEE, J. H. WALES, S. D. POLITYKA, AND R. O. SINNHUBER. 1970. The effect of dietary sterculic acid on the hepatic lipids of rainbow trout. Lipids 5: 80–84.

SINNHUBER, R. O., D. J. LEE, J. H. WALES, AND J. L. AYRES. 1968. Dietary factors and hepatoma in rainbow trout (*Salmo gairdneri*). II. Cocarcinogenesis by cyclopropenoid fatty acids and the effect of gossypol and altered lipids on aflatoxin-induced liver cancer. J. Natl. Cancer Inst. 41: 1293–1301.

SINNHUBER, R. O., J. H. WALES, R. H. ENGEBRECHT, D. L. AMEND, W. D. KRAY, J. L. AYRES, AND W. E. ASHTON. 1965. Aflatoxin in cottonseed meal and hepatoma in rainbow trout. Fed. Proc. 24: 627.

SINNHUBER, R. O., J. H. WALES, AND D. J. LEE. 1966. Cyclopropenoids, co-carcinogens for aflatoxin induced hepatoma in trout. Fed. Proc. 25: 55.

TAYLOR, S. L., M. W. MONTGOMERY, AND D. J. LEE. 1973. Liver dehydrogenase levels in rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids and aflatoxin B_1. J. Lipid Res. 14: 643–646.

WALES, J. H. 1967. Degeneration and regeneration of liver parenchyma accompanying hepatomagenesis, p. 56–60. *In* Trout Hepatoma Research Conference papers. U.S. Dep. of HEW and U.S. Dep. of the Interior, Washington.

WOLF, H., AND E. W. JACKSON. 1963. Hepatoma in rainbow trout: descriptive and experimental epidemiology. Science 142: 676–678.

WUNDER, W. 1971. Resistance and defense reaction of older rainbow trout against liver cancer (hepatoma) caused by aflatoxins. Arch. Fischereiwiss. 22: 51–64.

Mixing Within Experimental Enclosures: a Cautionary Note on the Limnocorral

F. M. BOYCE

Lakes Research Division
Canada Centre for Inland Waters, Burlington, Ont., L7R 4A6

BOYCE, F. M. 1974. Mixing within experimental enclosures: a cautionary note on the limnocorral. J. Fish. Res. Board Can. 31: 1400–1405.

A simple theoretical model was constructed to simulate heat transfer from a stratified lake surrounding an experimental enclosure constructed of a thin, flexible, vertical curtain. It was shown that for realistic dimensions of the apparatus and for modest horizontal turbulent diffusion processes within the enclosure, the vertical temperature profiles inside and outside the enclosure could be expected to be closely similar, and that the similarity was a result of horizontal heat transfers. In this situation, similarity of temperature profiles was not evidence of similarity of vertical transfer processes and experimental results within enclosures which depend on vertical transfers should be extrapolated with caution to the main body of the lake.

BOYCE, F. M. 1974. Mixing within experimental enclosures: a cautionary note on the limnocorral. J. Fish. Res. Board Can. 31: 1400–1405.

L'auteur construit un modèle théorique simple simulant le transfert de la chaleur d'un lac stratifié entourant une enceinte expérimentale formée d'un mince rideau vertical flexible. On démontre qu'afin d'obtenir des dimensions réalistes pour l'appareil et une diffusion turbulente horizontale modeste dans l'enceinte, on doit s'attendre que les profils verticaux de température à l'intérieur et à l'extérieur de l'enceinte se ressemblent étroitement, et que cette similarité soit le résultat de transferts horizontaux de chaleur. Dans une telle situation, la similarité des profils de température n'est pas le signe d'une similarité des procédés de transfert vertical, et toute extrapolation au corps principal du lac des résultats expérimentaux obtenus dans les enceintes qui dépendent de transferts verticaux doit être faite avec prudence.

Received May 25, 1973
Accepted April 19, 1974

Reçu le 25 mai, 1973
Accepté le 19 avril, 1974

IT has been found convenient for biological and chemical experimentation to wall off a portion of a lake with a vertical impermeable barrier usually made of a vinyl-impregnated fabric. The enclosure is open at the top (lake surface) to allow the passage of light and exchanges of heat, dissolved gases etc. with the atmosphere above. The arrangement is designed to simulate actual lake conditions within a controlled volume of water.

Printed in Canada (J3048)
Imprimé au Canada (J3048)

In a recent series of experiments, Hesslein and Quay (1973) have studied, among other things, vertical diffusion processes within an experimental enclosure. They also found essentially identical temperature profiles within and without the enclosure throughout their experiments. As commonly held theories of thermocline formation and decay usually consider only the vertical fluxes of heat and kinetic energy (Krauss and Turner 1967; Edwards and Darbyshire 1973), one is tempted to draw the conclusion from the similarity of temperature profiles that vertical turbulent transfer processes are similar as well, and more, that measurements of chemical and biological processes dependent on vertical transfers made within the enclosure (and made possible by the enclosure technique) can be extended to the lake as a whole.

Intuitively, one senses that vertical turbulence within the enclosure may well be different from that encountered outside, but short of actual measurements it is not obvious how to demonstrate this convincingly one way or the other. This note tackles the problem by showing that a close similarity between inside and outside temperature profiles can be accounted for entirely by horizontal fluxes of heat through the side walls. A theoretical model of the enclosure is developed incorporating the simplest geometry (and mathematics) which also constitutes a "worst case" situation for the proposition that similarly can be explained by horizontal transfers.

The model—The geometry of an enclosure situation is represented in the simplest way by a slab of water contained between two vertical planes, defined by $x = 0$ and $x = \mathrm{R}$ in a Cartesian coordinate system (Fig. 1). One plane ($x = 0$) is assumed to be thermally insulating and the other ($x = \mathrm{R}$) may be visualized as being constructed of the same material as the walls of the lake enclosure. Outside this barrier ($x > \mathrm{R}$) lies the main body of the lake which is assumed to be an infinite source of heat insofar as the enclosed mass of fluid is concerned. The vertical temperature profile in the lake is imposed and consists of a warm upper layer lying atop a cool lower layer with a step discontinuity in temperature between them. This step (thermocline) moves downwards at a speed C, the value of which is chosen to lie towards the extreme range of speeds of descent of the thermocline under conditions of simultaneous heating from the surface and wind mixing.

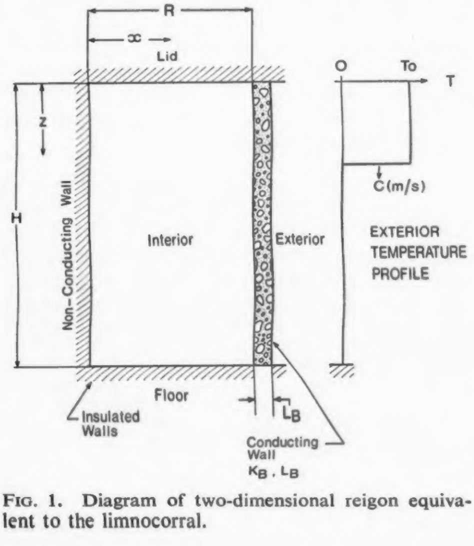


FIG. 1. Diagram of two-dimensional reigon equivalent to the limnocorral.

The enclosure being open at the top, a complete model would require the specification of the fluxes of heat across the water surface into the enclosure. In the case studied here, however, the boundaries at the top and bottom of the slab of fluid were assumed to be nonconducting also. This restriction means that the only pathway of heat from the lake to the enclosed slab is horizontally via the curtain, and thus comprises a more severe test of the hypothesis that horizontal diffusion is the dominant transfer process. This measure also simplifies the mathematical analysis.

The problem may be formulated mathematically in the following way. Let $\mathrm{T}(x,y,t)$ be the temperature field in the enclosure region which is described by $0 \leq x \leq \mathrm{R}, 0 \leq z \leq \mathrm{H}$. x is the horizontal coordinate and R is the width of the enclosed region. R may be viewed as a scale length for the cross sectional diameter of limnocorrals in general. z is the vertical coordinate taken positive downwards and H is the depth of the water column. H is not a critical dimension to the problem but is chosen so that the aspect ratio H:R of the enclosure is about what one would encounter in the field. The process of thermal diffusion in the enclosure is represented by the equation

$$\frac{\partial \mathrm{T}}{\partial t} = \mathrm{K_H} \frac{\partial^2 \mathrm{T}}{\partial x^2} + \mathrm{K}_v \frac{\partial^2 \mathrm{T}}{\partial z^2}; \quad \begin{array}{c} 0 \leq x \leq \mathrm{R} \\ 0 \leq z \leq \mathrm{H} \end{array} \tag{1}$$

where $\mathrm{K_H}$ and K_v are the horizontal and vertical (constant) coefficients of thermal diffusivity, respectively. The boundary conditions (no heat flux) at the nonconducting walls are written

$$\begin{aligned} \frac{\partial \mathrm{T}}{\partial z} &= 0 \qquad \text{at } z = 0 \text{ and } z = \mathrm{H} \\ \frac{\partial T}{\partial x} &= 0 \qquad \text{at } x = 0 \end{aligned} \tag{2}$$

The barrier material ($x = \mathrm{R}$) is assumed to be thin enough so that the heat flow through it is proportional to the temperature difference between the fluid in the enclosure against the barrier and the temperature in the lake outside, the latter represented by $\mathrm{T}_e(z,t)$. The boundary condition at $x = \mathrm{R}$ is the written

$$\mathrm{K_H} \frac{\partial T}{\partial x} = \left(\frac{\mathrm{K_B}}{\mathrm{L_B}}\right) (\mathrm{T_e}(z, t) - \mathrm{T}(\mathrm{R}, z, t)) \text{ at } x = \mathrm{R} \tag{3}$$

where K_B and L_B are the thermal diffusivity and the thickness of the barrier material, respectively. The initial conditions are given by

$$T(x,z,t) = T_e(z,t) = 0 \text{ at } t = 0 \qquad (4)$$

Finally, the external temperature field is defined as a moving step function

$$\begin{aligned} T_e(z,t) &= T_o \; ; \; z \leq z_t \\ T_e(z,t) &= 0 \; ; \; z > z_t \qquad (5) \\ z_t &= Ct \; ; \; 0 < t < \frac{H}{C} \end{aligned}$$

z_t is the depth of the thermocline and the thermocline is assumed to progress vertically through the region at a uniform speed, C.

It will be convenient to cast equations (1) to (5) into nondimensional form. We define scales for the independent variables x, z, and t (R, Z, τ) such that

$$\begin{aligned} t &= \tau\, t^* \\ x &= R\, x^* \qquad (6) \\ z &= Z\, z^* \end{aligned}$$

which set into (2) yields

$$\frac{K_H}{R^2}\left(\frac{\partial^2 T}{\partial x^{*2}}\right) + \frac{K_v}{Z^2}\left(\frac{\partial^2 T}{\partial z^{*2}}\right) = \frac{1}{\tau}\frac{\partial T}{\partial t^*} \qquad (7)$$

We further impose

$$\frac{K_H}{R^2} = \frac{K_v}{Z^2} = \frac{1}{\tau} \qquad (8)$$

from which we have

$$\begin{aligned} \tau &= R^2/K_H \\ Z &= R\left(\frac{K_v}{K_H}\right)^{\frac{1}{2}} \qquad (9) \end{aligned}$$

The physical shape of the region is defined by $h = H/R$; thus z^* ranges from 0 to $h(\frac{K_v}{K_H})^{-\frac{1}{2}} = Z^*_m$ and t^* ranges from 0 to $\frac{H K_H}{R^2 C} = \frac{h K_H}{RC} = \gamma$

The boundary condition (3) becomes

$$\frac{\partial T}{\partial x^*} = \left(\frac{K_B}{L_B} \cdot \frac{R}{K_H}\right)(T_e(z^*, t^*) - T) \qquad (10)$$

$$x^* = 1$$

The nondimensional form of equations (2) to (5) is

$$\frac{\partial T}{\partial t^*} = \frac{\partial^2 T}{\partial x^{*2}} + \frac{\partial^2 T}{\partial z^{*2}} \qquad \begin{aligned} &0 \leq x^* \leq 1 \\ &0 \leq z^* \leq Z^*_m \\ &0 \leq t^* \leq \gamma \end{aligned} \qquad (11)$$

$$\frac{\partial T}{\partial x^*} = \beta(T_e - T) \; ; \; x^* = 1 \qquad (12)$$

$$\text{where } \beta = \left(\frac{K_B\, R}{K_H\, L_B}\right)$$

$$\frac{\partial T}{\partial z^*} = 0 \; ; \; \begin{matrix} z = 0 \\ z = z^*_m \end{matrix} \, , \; \frac{\partial T}{\partial x^*} = 0 \; ; \; x^* = 0 \qquad (13)$$

The various parameters are defined in Table 1.

For the case in hand we choose approximate values

$R = 200$ cm
$H = 20\ m = 2 \times 10^3$ cm
$C = 10^{-4}$ cm/s $= .86$ m/day
(maximum rate)
$K_B = 3 \times 10^{-4}$ cm^2/s
$L_B = 0.1$ cm
$K_v = 12 \times 10^{-4}$ cm^2/s (molecular value of thermal diffusivity of water)

We express K_H as a multiple of K_v

$$K_H = D\, K_v$$

D = 1 corresponds to a limiting case of molecular diffusion in both the vertical and horizontal directions. In the open lake environment a horizontal eddy diffusivity acting at the scale of the limnocorral is of the order of 10^2 cm^2/s (Murthy 1970; Okubo 1971). This value corresponds to a value of D in excess of 10^5. Solutions of the equations were computed for values of D ranging from 1 to 10^3 (See *Discussion*).

Solution — The solution of equations (11) to (13) proceeds in a straightforward if tedious fashion using first a Laplace transformation of the time variable then a Green's function technique to deal with the nonhomogeneous boundary condition (12). The imposed exterior temperature is expanded as a Fourier series in the vertical eigen-functions of the Green's function

$$T_e(z^*, t^*) = T_o\left(\frac{t^*}{\gamma} + \frac{2}{\pi}\sum_{j=1}^{\infty}\frac{\sin\left(\frac{j\pi t^*}{\gamma}\right)}{j} \cos\left(j\frac{\pi z^*}{Z^*m}\right)\right) \qquad (14)$$

The temperature at $x^* = 0$ (the point farthest away from the conducting barrier) is given by

$$\mathrm{T}(0, z^*, t^*) = \mathrm{T}_o\left[\sum_{i=1}^{\infty} \frac{m_i}{q_i^4}(e^{-q_i^2 t^*} - 1 + q_i^2 t^*) \right. \tag{15}$$

$$+ 2\sum_{i=1}^{\infty}\sum_{j=1}^{\infty} \frac{\mathrm{M}_i \cos\left(\frac{j\pi z^*}{Z^*m}\right)}{j\frac{\pi}{\gamma}\sqrt{\Omega ij^2 + \left(j\frac{\pi}{\gamma}\right)^2}}$$

$$\left.(\sin(j\frac{\pi t^*}{\gamma} - \theta_{ij}) + e^{-\Omega_{ij}t^*}\sin\theta_{ij})\right]$$

where

$$q_i \tan q_i = \beta \qquad i = 1, 2, \ldots$$

$$\Omega_{ij} = q_i^2 + \left(\frac{j\pi}{Z_m^*}\right)^2$$

$$\mathrm{M}_i = \frac{2\beta\, q_i \cos q_i}{\gamma(q_i + \sin q_i \cos q_i)}$$

$$\theta_{ij} = \tan^{-1}\left(\frac{(j\frac{\pi}{\gamma})}{\Omega_{ij}}\right)$$

Equation (15) is evaluated at $t^* = \frac{\gamma}{2}$ (thermocline at middepth) with $\mathrm{T}o = 1$. The single sum is the mean temperature, and the double sum represents the spatially varying component. The computations are carried to 20 terms in i and j each, and the results are compared with equation (14) truncated similarly at 20 terms. The computations are carried out for D = 1, 10, 100, and 1000 (Fig. 2).

The truncation of the Fourier series at 20 terms causes the overshoots ($\mathrm{T}/\mathrm{T}o > 1$ and $\mathrm{T}/\mathrm{T}o < 0$). In any case, the exterior temperature may be regarded as the true forcing function and is adequate for our purposes.

The solutions in the interior ($x = 0$) for D = 1 and D = 10 exhibit both time lag and vertical smoothing. The solutions for D = 100 and D = 1000 correspond to the exterior temperature with the latter solution being essentially identical to the exterior solution at the resolution of Fig. 2. The error in thermocline depth for D = 100 (depth of the 0.5 isotherm) is 22 cm for the case considered. For D = 1000, the error is reduced to 9 cm.

TABLE 1. Definition of parameters. (See Fig. 1 for definition of dimensions).

Parameter	Function	Formula
h	defines shape of region	$h = \mathrm{H}/\mathrm{R}$
D	ratio of horizontal to vertical (molecular) diffusion coefficients	$\mathrm{D} = \frac{\mathrm{K_H}}{\mathrm{K_v}}$
	basic time scale	$\tau = \frac{\mathrm{R}^2}{\mathrm{K_H}} = \frac{\mathrm{R}^2}{\mathrm{DK_v}}$
Z	vertical length scale	$\mathrm{Z} = \frac{\mathrm{R}}{\sqrt{\mathrm{D}}}$
Z_m^*	range of nondimensional vertical coordinate $0 \leq z^* \leq \mathrm{Z}_m^*$	$\mathrm{Z}_m^* = h\sqrt{\mathrm{D}}$
τ_s	Time taken for thermocline to progress through the region	$\tau_s = \frac{\mathrm{H}}{\mathrm{C}}$
γ	Ratio of τ_s to τ, range of t^* $0 \leq t^* \leq \gamma$	$\gamma = \frac{\tau_s}{\tau} = \frac{\mathrm{H}}{\mathrm{C}}\frac{\mathrm{K_H}}{\mathrm{R}^2}$

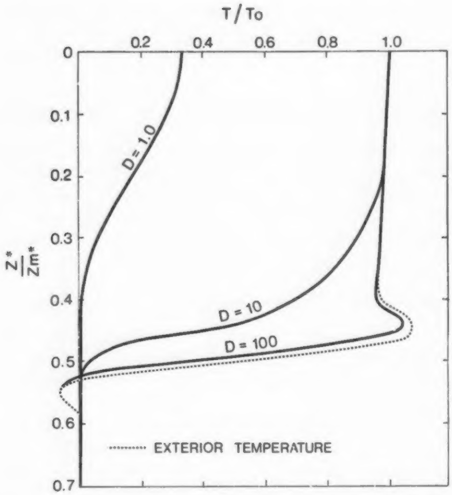


FIG. 2. Solutions to equations (11), (12), and (13) at $x = 0$, $t = \tau_z/2$, for D = 1, 10, and 100. The exterior temperature profile at $x = R$, $t = \tau_z/2$ is shown as a dashed line.

Discussion and conclusions — By choosing D as the independent parameter, with K_v fixed at the value of molecular thermal diffusivity, we are exploring the effects of varying horizontal diffusivity on the temperature structure within the column. If the vertical diffusivity were larger, and if the solution included a heat flux from the surface, one would expect a diminished sensitivity to the value of K_H. The problem is thus consistently posed as a worst case for the premise that conduction through the barrier and subsequent horizontal diffusion within the enclosure can account for the similarity between interior and exterior temperature profiles.

The vertically hanging but flexible barrier must transmit differently the vertical and horizontal components of turbulence existing in the lake. Momentum from horizontal pulsations can be transmitted, albeit attenuated, to the interior by virtue of the flexibility of the barrier in directions perpendicular to itself, but vertical motions must set up shear forces in the plane of the barrier which are resisted by the weight of the barrier and the forces exerted by its supporting armature and so cannot be transmitted into the interior. Without making actual measurements of turbulence levels in the enclosure, it is impossible to assess the attenuation of horizontal turbulence. However, if a value of D of 10^5 is obtained for a K_H representative of the open lake, a value of D of 10^3 in the enclosure would seem to be a conservative estimate and to allow for a considerable attenuation.

Because of the complicated way the parameters enter into the solutions, (transcendental equations for the x dependent portion) the only exact way of evaluating the effects of changes in the parameters is to recompute the entire solution. This has been done for R = 400 cm, a doubling of the effective radius of the enclosure, with the other parameters being held the same as before. A solution comparable to the one previously obtained for D = 100 now requires a value of D closer to 1000, and assuming an order of magnitude increase in D for each doubling of R the argument would still apply at R = 16 m.[1]

The weight of accumulated evidence on the physics of the formation and maintenance of a mixed upper layer (Boyce 1974) suggests that a significant portion of turbulent energy in the upper layer is provided at times by the surface wave field, particularly through the mechanism of forward breaking. Pollard et al. (1973) propose that the principal mechanism for turbulent entrainment at the bottom of the upper mixed layer in a large body of water is dynamic instability due to the interaction of the mean wind driven current in the upper layer and the density change across the thermocline. The effects of both surface waves and mean currents would be absent or severely reduced in an artificial enclosure, and this argument reinforces our suspicions that vertical turbulence regimes within an enclosure differ from those outside.

From the theoretical argument presented in the main body of this note it is concluded that similar temperature profile inside and outside enclosures made of thin flexible curtains are to be expected but that this similarity is not to be construed as evidence of a similarity of the vertical transfer processes. Until more definitive evidence is collected, and the above paragraph suggests that it will not be, a cautious approach to enclosure experiments depending on vertical transfers is warranted.

BOYCE, F. M. 1974. Some aspects of Great Lakes physics of importance to biology and chemistry. J. Fish. Res. Board Can. 31: 689–730.

EDWARDS, A., AND J. DARBYSHIRE. 1973. Models of a lacustrine thermocline. Extrait des mémoires de la Société Royale des Sciences de Liege. 6^e Série, Tome 4.

---

[1] The larger the effective diameter of the enclosure, the less attenuation one expects of horizontal turbulence at scales comparable to the diameter because the changes in curvature of the fabric induced by the turbulence will decrease.

HESSLEIN, R., AND P. QUAY. 1973. Vertical eddy diffusion in the thermocline of a small stratified lake. J. Fish. Res. Board Can. 30: 1491–1500.

KRAUS, E. B., AND J. S. TURNER. 1967. A one-dimensional model of the seasonal thermocline II. The general theory and its consequences. Tellus 19. 98–106.

MURTHY, C. R. 1970. An experimental study of horizontal diffusion in Lake Ontario. Proc. 13th Conf. Lakes Res. 1970. Int. Assoc. Great Lakes Res. p. 477–489.

OKUBO, A. 1971. Oceanic diffusion diagrams. Deep Sea Res. 18: 789–802.

PHILLIPS, O. M. 1966. The dynamics of the upper ocean. Cambridge Univ. Press, Cambridge. 261 p.

POLLARD, R. T., P. B. RHINES, AND R. O. R. Y. THOMPSON. 1973. The deepening of the wind mixed layer. Geophys. Fluid Dyn. 3: 381–404.

Phosphoglucomutase Polymorphism in Brown Shrimp, *Penaeus aztecus*[1]

R. R. PROCTOR, K. T. MARVIN, L. M. LANSFORD, AND R. C. BENTON

National Marine Fisheries Service, Gulf Coastal Fisheries Center
Galveston Laboratory, Galveston, Tex. 77550, USA

PROCTOR, R. R., K. T. MARVIN, L. M. LANSFORD, AND R. C. BENTON. 1974. Phosphoglucomutase polymorphism in brown shrimp, *Penaeus aztecus*. J. Fish. Res. Board Can. 31: 1405–1407.

Eight phenotypes of phosphoglucomutase (PGM) were detected in abdominal muscle extracts from brown shrimp (*Penaeus aztecus*) by starch-gel electrophoresis. The observed phenotypes were assumed to be under the control of five allelic genes. This assumption was supported by the observed distribution of phenotypes.

There were no significant differences in PGM phenotype distribution between sexes or among samples of shrimp taken from Galveston Bay, Texas, and Vermilion and Barataria bays, Louisiana.

PROCTOR, R. R., K. T. MARVIN, L. M. LANSFORD, AND R. C. BENTON. 1974. Phosphoglucomutase polymorphism in brown shrimp, *Penaeus aztecus*. J. Fish. Res. Board Can. 31: 1405–1407.

Nous avons décelé, par électrophorèse de gel amidon, huit phénotypes de phosphoglucomutase (PGM) dans des extraits du muscle abdominal de la crevette grise (*Penaeus aztecus*). Nous supposons que les phénotypes observés sont sous le contrôle de cinq gènes allèles. La répartition des phénotypes, telle qu'observée, confirme cette hypothèse.

Il n'y a pas de différences significatives dans la répartition des phénotypes de PGM entre les sexes ou entre les échantillons provenant de la baie Galveston, Texas, et ceux des baies Vermilion et Barataria, Louisiane.

Received December 31, 1973
Accepted April 16, 1974

Reçu le 31 décembre, 1973
Accepté le 16 avril, 1974

PHOSPHOGLUCOMUTASE (PGM) is a phosphotransferase that catalyses a reaction converting glucose-1-phosphate to glucose-6-phosphate. Genetic variation of PGM was first reported in man by Spencer et al. (1964). Hopkinson and Harris (1965, 1968) reported three independent loci in man, each locus having two or more allelic forms.

Genetic variants of PGM also have been described in several teleosts. A two-allele system was reported in rainbow trout (*Salmo gairdneri*) by Roberts et al. (1969). Lush (1969) presented evidence for two PGM loci (one was polymorphic) in Atlantic herring (*Clupea harengus*). A two-allele PGM system was also reported in sockeye salmon (*Oncorhynchus nerka*) by Utter and Hodgins (1970).

The objective of our research was to find genetically controlled biochemical variants that can be

---

[1] Contribution No. 377, from the National Marine Fisheries Service, Gulf Coastal Fisheries Center, Galveston Laboratory, Galveston, Tex. 77550, MARMAP Contribution No. 3, USA.

Printed in Canada (J3255)
Imprimé au Canada (J3255)

used to determine whether or not different stocks exist within each of the three most important commercial shrimp species (*Penaeus aztecus*, *P. setiferus*, and *P. duorarum*) in the Gulf of Mexico. This paper describes PGM polymorphism in brown shrimp (*P. aztecus*) and presents data on distribution of phenotypes and frequency of alleles for brown shrimp taken from three locations in the northwestern Gulf of Mexico.

Methods — Juvenile brown shrimp between 60 and 100 mm in total length (tip of rostrum to tip of telson) were collected from three locations: Galveston Bay, Texas; Vermilion Bay, Louisiana; and Barataria Bay, Louisiana (Fig. 1). Two hundred specimens (100 of each sex) from each location were frozen immediately with dry ice for transportation to the laboratory where they were stored at –80 C until analyzed.

Equal parts (about 0.5 cm^3 each) of partially thawed abdominal muscle and phosphate buffered physiological saline (Johnson et al. 1972) were blended to extract PGM from each specimen. A glass rod was used to grind and mix the muscle and saline in a 10- by 75-mm culture tube, and the mixture was centrifuged at 31,000 $\times g$ for 10 min at 5 C. Without further treatment, the cleared extract was drawn into a 4- by 6-mm Whatman No. 1 filter paper insert. The insert was blotted lightly, then placed with inserts representing other specimens in starch-gel prepared according to Kristjannson (1963). A buffer system by Ridgway et al. (1970) was used, and horizontal starch-gel electrophoresis and subsequent staining of PGM followed the method described by Johnson et al. (1972).

Results — Zymograms of abdominal muscle extracts exhibited a single region of PGM activity occupied by five anodal bands labelled a, b, c, d, and e (Fig. 2). These bands are assumed to be under the control of five codominant allelic genes, designated herein as PGM^a through PGM^e. This assumption was supported by observed distributions of one- and two-banded phenotypes (Table 1) which did not differ significantly from expected distributions based upon Hardy-Weinberg equilibrium (Stern 1943). Eight of the 15 possible phenotypes were detected, and their observed distribution in samples from the three bays is shown in Table 1. Our failure to detect the seven remaining phenotypes was not surprising, because PGM alleles a, d, and e were rare (Table 1).

A three-banded pattern (Fig. 2) observed in one female shrimp from Galveston Bay was counted as a bd phenotype. The extra band may have resulted from a nongenetic modification of one of the bands. Such conformational isozymes are common for PGM in vertebrates (Brewer 1970). An alternate explanation for the extra band would be that this particular individual was trisomic for the chromosomes carrying the PGM locus, with each chromosome carrying a different allele. A similar situation for LDH in brook trout (*Salvelinus fontinalis*) was discussed by Davisson et al. (1972).

The distribution of observed phenotypes was not significantly dependent on sex (Table 1); therefore, the samples of males and females from each bay were combined for the comparison among the three bays (Table 1). There was no significant difference in phenotype distribution among the three bays (Table 1). This means that separate stocks of brown shrimp were not detected in these

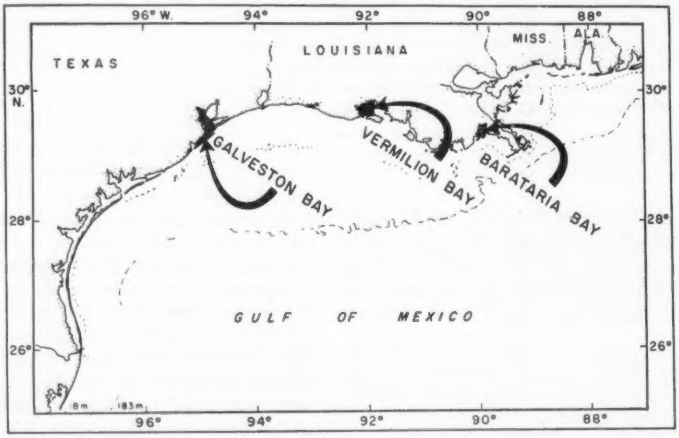


FIG. 1. Bays from which brown shrimp (*Penaeus aztecus*) samples were collected.

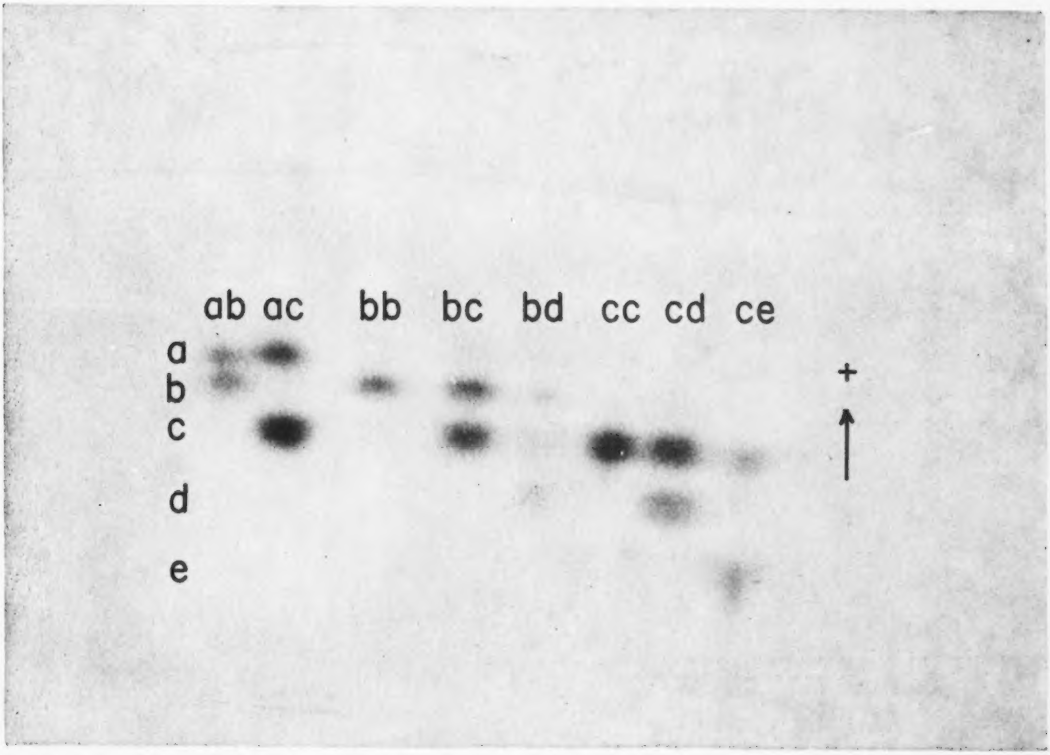

ab
ac
bb
bc
bd
cc
cd
ce
a
b
c
d
e
+

FIG. 2. Bands in starch-gel illustrating five PGM alleles and eight PGM phenotypes detected in brown shrimp. Direction (↑) of protein migration toward the anode (+) is shown.

Proctor et al. — J. Fish. Res Board Can.

TABLE 1. Distribution (number of specimens) of eight PGM phenotypes and frequency of PGM alleles in samples of brown shrimp (*Penaeus aztecus*) from Galveston Bay, Texas, and Vermilion and Barataria bays, Louisiana.

Location	Phenotypes								Alleles				
	ab	ac	bb	bc	bd	cc	cd	ce	a	b	c	d	e
Galveston Bay	1	1	9	74	3	110	2	0	0.0050	0.2400	0.7425	0.0125	0.0000
Barataria Bay	0	1	5	68	2	119	4	1	0.0025	0.2000	0.7800	0.0150	0.0025
Vermilion Bay	0	0	8	69	0	116	6	1	0.0000	0.2125	0.7700	0.0150	0.0025

Comparison of phenotype distributions	df	Chi* square
Males vs. females (Galveston Bay)	2	4.109
Males vs. females (Vermilion Bay)	2	0.745
Males vs. females (Barataria Bay)	2	0.151
Comparison among the three bays (sexes combined)	4	2.642

*Frequencies for phenotypes containing a, b, d, and e bands were pooled under one category for the Chi square calculations.

three bays. It is possible, however, that sample size was too small to detect a significant difference in phenotype distribution among the three bays, under the assumption that such slight differences were real.

To our knowledge this is the first report of PGM polymorphism in a penaeid shrimp. We believe that the PGM genetic system described herein has considerable potential as a tool in population studies of brown shrimp and other *Penaeus* spp. in the Gulf of Mexico. As no differences in PGM phenotype distribution were detected among samples from Galveston Bay, Vermilion Bay, and Barataria Bay, future comparisons will include samples from areas separated by greater distances and by presumed barriers to shrimp migration (e.g. discharge from the mouth of the Mississippi River).

Acknowledgments — We are grateful to the Division of Oysters, Water Bottoms and Seafood and the Refuge Division of the Louisiana Wild Life and Fisheries Commission for assistance in collection of samples in Louisiana. Dr F. M. Utter and Dr A. G. Johnson, National Marine Fisheries Service, Northwest Fisheries Center, Seattle, Wash., provided guidance concerning electrophoretic techniques and reviewed the manuscript.

BREWER, G. J. 1970. Terminology and definitions, p. 8–13. *In* G. J. Brewer, An introduction to isozyme techniques. Academic Press Inc., New York, N.Y.

DAVISSON, M. T., J. E. WRIGHT, AND L. M. ATHERTON. 1972. Centric fusion and trisomy for the LDH-B locus in brook trout, *Salvelinus fontinalis*. Science 178: 992–994.

HOPKINSON, D. A., AND H. HARRIS. 1965. Evidence for a second "structural" locus determining human phosphoglucomutase. Nature (Lond.) 208: 410–412.

1968. A third phosphoglucomutase locus in man. Ann. Hum. Genet. 31: 359–367.

JOHNSON, A. G., F. M. UTTER, AND H. O. HODGINS. 1972. An electrophoretic investigation of the family Scorpaenidae. U.S. Dep. Commer., Natl. Oceanic Atmos. Admin. Fish. Bull. 70: 403–413.

KRISTJANSSON, F. K. 1963. Genetic control of two pre-albumins in pigs. Genetics 48: 1059–1063.

LUSH, I. E. 1969. Polymorphism of a phosphoglucomutase isoenzyme in the herring (*Clupea harengus*). Comp. Biochem. Physiol. 30: 391–395.

RIDGWAY, G. J., S. W. SHERBURN, AND R. D. LEWIS. 1970. Polymorphism in the esterases of Atlantic herring. Trans. Am. Fish. Soc. 99: 147–151.

ROBERTS, F. L., J. F. WOHNUS, AND S. OHNO. 1969. Phosphoglucomutase polymorphism in the rainbow trout, *Salmo gairdneri*. Experientia 25: 1109–1110.

SPENCER, N., D. A. HOPKINSON, AND H. HARRIS. 1964. Phosphoglucomutase polymorphism in man. Nature (Lond.) 204: 742–745.

STERN, C. 1943. The Hardy-Weinberg Law. Science 97: 137–138.

UTTER, F. M., AND H. O. HODGINS. 1970. Phosphoglucomutase polymorphism in sockeye salmon. Comp. Biochem. Physiol. 36: 195–199.

An Isolated Population of Arctic Char (*Salvelinus alpinus*) Inhabiting a Warm Mineral Spring Above a Waterfall at Cache Creek, Northwest Territories

P. McCart and H. Bain

Aquatic Environments Limited, Crossfield, Alta.

McCart, P., and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. J. Fish. Res. Board Can. 31: 1408–1414.

Cache Creek Spring provides an unusual overwintering habitat for a population of Arctic char (*Salvelinus alpinus*) isolated above a falls impassable to fish moving upstream. During winter, water temperatures (14–16 C) and dissolved solid concentrations (approximately 2600 ppm) are high and oxygen concentrations (0.2–6.8 ppm) are low. Arctic char in the springs differ meristically from those downstream of the falls. The former have significantly more gillrakers (mean 21.7 compared with 21.1) and parr marks (15.1 and 13.5) but fewer pyloric caeca (26.0 and 28.2) and vertebrae (64.7 and 67.1) than the latter. Growth appears similar to that of char inhabiting more typical spring habitats.

McCart, P., and H. Bain. 1974. An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories. J. Fish. Res. Board Can. 31: 1408-1414.

La source du ruisseau Cache fournit un habitat hivernal inusité à une population d'ombles chevaliers (*Salvelinus alpinus*) isolée en amont d'une chute infranchissable pour les poissons qui remontent le cours d'eau. En hiver, les températures de l'eau (14–16 C) et les teneurs en solides dissous (environ 2600 ppm) sont élevées, et les teneurs en oxygène (0.2–6.8 ppm) sont basses. Les caractères numériques des ombles chevaliers de la source diffèrent de ceux des ombles se trouvant en aval des chutes. Les premiers ont des comptes significativement plus élevés de branchicténies (moyenne de 21.7 comparativement à 21.1) et de marques de tacon (15.1 et 13.5), mais moins élevés de caeca pyloriques (26.0 et 28.2) et de vertèbres (64.7 et 67.1) que les derniers. La croissance semble être semblable à celle d'ombles habitant des sources plus typiques.

Received February 14, 1974
Accepted April 19, 1974

Reçu le 14 février, 1974
Accepté le 19 avril, 1974

This paper describes an isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring in the headwaters of Cache Creek (Little Fish River) in the Northwest Territories. Populations of Arctic char inhabiting springs have been reported elsewhere in the western Arctic (McCart and Craig 1973; McCart et al. 1972). However, during the winter the Cache Creek population occupies a habitat characterized by high temperature, high dissolved solids content, and low oxygen levels, very different from that described for other spring populations. The latter occupy springs in which winter temperatures and dissolved solid concentrations are low and oxygen levels are high. An analysis of the life history of the population indicates that it has a growth rate comparable to that of Arctic char occupying more typical spring habitats. The conditions under which the Cache Creek population survives and apparently thrives, indicate a wide tolerance to environmental extremes. Of particular interest, especially to those interested in the impact of human disturbance on arctic freshwater fishes, is the evident tolerance of the population to low oxygen concentrations.

Materials and methods — Samples of fish were taken by seine and electrofisher on November 11, 1972 (12 fish) and on April 19 (50 fish), May 10 (70 fish including 30 young-of-the-year), and July 4, 1973 (30 fish, all young-of-the-year). The methods of life history analysis were those described by McCart and Craig (1973). Gillraker and pyloric caeca counts were determined as described by McCart and Craig (1971). Counts of parr marks included all marks which traversed the lateral line on the left side of the body. Vertebral counts were made from radiographs and include vertebrae within the hypural complex.

Oxygen concentrations were determined in the field with a Hach Kit (model DR-EL) and water velocities

Printed in Canada (J3320)
Imprimé au Canada (J3320)

for discharge estimates were determined with a Gurley Pygmy Current Meter.

Description of the study area — Cache Creek (Little Fish River) is a tributary of the Fish River (Big Fish River) and originates in the Richardson Mountains in the Yukon and Northwest Territories (Fig. 1). Cache Creek Spring is located just inside the Northwest Territories on the westernmost of the stream's three major branches (approximately 68°33′N and 136° 17′W). The spring waters originate from several orifices. There is a series of waterfalls approximately 400 m downstream. The highest of the falls is approximately 8–10 m and prohibits fish movement upstream. The total discharge from spring sources above the falls was estimated at 0.31 m^3/s on November 11, 1972 and 0.35 m^3/s on April 19, 1973.

Dr R. O. van Everdingen (1973a, b) has carried out detailed chemical analysis of water samples supplied by the authors (Table 1). Cache Creek Spring water is warm, with a high dissolved solid content, high specific conductance, and very low oxygen level. The water has high concentrations of Na, K, and Cl and a high Na:K ratio (47:1). The high temperature and low oxygen are indicative of a long (or slow), deep flow system. R. Mutch (personal communication) has suggested that the high dissolved solids content may result from the solution of evaporitic halite at depth.

The chemical characteristics of the water are unique among those of springs in the western Arctic with resident populations of Arctic char (Table 1). The concentrations of the major ions exceed the means (and with one exception, the upper range) of concentrations for any of the other 15 springs for which data are presently available, and the water temperatures are the highest yet recorded.

In late winter (May 10, 1973), before spring breakup had occurred in this area, high water temperature and low dissolved oxygen concentrations were found throughout the area upstream and for some distance downstream of the falls (Table 2). Some earlier data, obtained April 19, 1973, were comparable: at the major orifice the water temperature was 15 C and oxygen concentration 0.5 ppm (5% saturation); 70 m downstream the temperature was 14 C and the oxygen concentration 4 ppm (40% saturation).

The area upstream of the falls was visited again June 10, 1973 during the spring freshet. No discharge measurements were made but discharge from the springs was only a small proportion (estimated at less than 5%) of the total. Water temperature was the same (3.5 C) upstream and downstream of the major orifice. The dissolved oxygen concentration just downstream of the orifice was 11.8 ppm (92% saturation).

The fish population — Although grayling (*Thymallus arcticus*) occur together with Arctic char downstream of the falls, only Arctic char have been taken upstream. The Arctic char above and below the falls appear to constitute two distinct populations. Their gillraker and pyloric caeca counts (Table 3) indicate that fish from both localities are of the western Arctic–Bering Sea type (McPhail 1961; McCart and Craig 1971). However, *t*-test comparisons of means indicate that there are significant differences ($P < 0.05$) between the upstream and downstream populations in these characters as well as highly significant ($P < 0.01$) differences in vertebral counts and numbers of parr marks. The life history data described below apply only to the isolated population above the falls.

On May 10, 1973, Arctic char were distributed from the headwater spring downstream to the falls

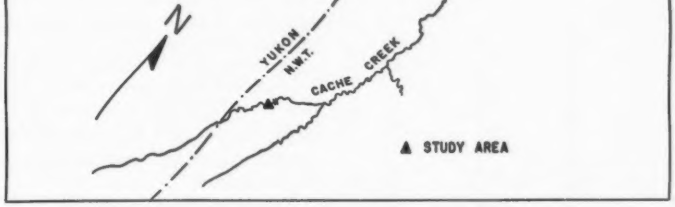


FIG. 1. Location of study area on Cache Creek, N.W.T.

TABLE 1. Physical and chemical characteristics of water samples from Cache Creek Spring compared with those of 15 other springs inhabited by Arctic char (*Salvelinus alpinus*) in Beaufort Sea drainages in the Yukon Territory and Alaska. Data for the latter from van Everdingen (1973 a, b) and P. McCart and P. Craig (unpublished data). Where unspecified, units are mg/liter. Temperature and oxygen values were determined in the field, all others in the laboratory. SD is standard deviation.

	Cache Creek Spring Date		15 other springs		
	Nov. 11, 1972	Apr. 19, 1973	Mean	Range	SD
Temp C	15.5	15.0	2.7	0.0–12.5	3.0
Conductivity μmho/cm at 25 C	4546	4531	297	235–454	50.7
pH units	7.8	7.7	7.9	7.6–8.2	0.2
Dissolved oxygen	2.0	0.5	10.5	5.0–14.5	3.6
Ca	95.0	102.0	44.6	36.3–58.5	6.5
Mg	22.1	17.8	8.2	4.0–19.0	3.6
Na	824	760	2.5	0.3–19.0	4.7
K	17.5	16.0	0.4	0.2–1.4	0.4
Na/K	47.1	47.5	3.0	0.5–12.7	5.1
HCO_3	267	272	148.6	119.1–201.3	21.4
SO_4	417	367	21.1	9.1–62.8	12.7
Cl	1036	1030	1.4	0.1–14.8	3.7
Total dissolved solids	2698.1	2585.0	281.2	191.7–334.0	36.9

with a major concentration, estimated at more than 500 juvenile and adult fish as well as an abundance of young-of-the-year, about midway between the two points. When the area was visited July 4, fish were widely distributed through the same stretch and adults particularly seemed much less abundant. They are thought to have moved upstream to feed in areas dry during the winter.

TABLE 2. Changes in water temperature and oxygen concentration downstream of major orifice, Cache Creek Spring, May 10, 1973. Data were obtained at midstream. The sample site at 650 m is approximately 250 m downstream of the impassable falls.

Approx. distance downstream of major orifice (*m*)	Water temp (*C*)	Oxygen concn (*ppm*)	% Saturation
0	16	0.2	2
7	15	1.2	12
15	15	1.2	12
24	15	2.1	21
40	15	2.2	22
75	15	3.6	37
150	15	4.4	45
300	14	6.8	67
650	11	8.8	82

A peculiarity of the Cache Creek Spring populations is the occurrence of protuberant eyes among fish captured in the late winter. In some cases, the eyes were grossly distended and it is likely that the fish were blind. On May 10, 1973, the overall incidence was 30% in a sample of 40 with the greatest incidence (60%) in the sample of 10 taken in the immediate vicinity of the major orifice. In addition, some fish had swellings along the dorsal and ventral surfaces of their bodies. The symptoms appear to be those of gas bubble disease. Davies (1965) has described this condition and listed a variety of causes including supersaturation of gases (either N_2 or O_2), accumulations of serous fluids, parasitic infestations, and bacterial infections. The reasons for the occurrence of gas bubble disease among Cache Creek char are not known. However, nitrogen gas is common in spring waters (van Everdingen 1972) and may be involved in this instance. There were no obvious abnormalities among any of the fry examined May 10 or July 4, 1973.

The spawning period of the population is unknown but appears to be in late fall or early winter. Eleven mature females taken November 11, 1972 contained large, mature eggs but these were tightly bound by ovarian membranes and spawning was still sometime in the future.

The period from spawning to emergence appears to be short. Fry collected May 10, 1973 were already

TABLE 3. Comparison of meristic characters for Arctic char captured upstream and downstream of falls on Cache Creek, Yukon Territory. Data include sample size (N), mean, standard deviation, and range, along with results of t-test analyses.

	Fork length				Gillrakers				Pyloric caeca				Vertebrae				Parr marks			
Locality	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range	N	Mean	SD	Range
Above falls	30	211.0	(21.39)	171–244	30	21.7	(0.97)	20–24	29	26.0	(2.66)	21–32	29	64.7	(1.72)	58–67	31	15.1	(0.99)	13–17
Below falls	38	194.6	(15.48)	127–422	38	21.1	(1.30)	19–24	37	28.2	(3.68)	22–35	31	67.1	(0.95)	66–69	29	13.5	(1.19)	12–17
t	DF = 66 $t = 0.9066$				DF = 66 $t = 2.1417$				DF = 64 $t = 2.6461$				DF = 58 $t = 6.744$				DF = 58 $t = 5.69$			
P	$P > 0.05$				$P < 0.05$				$P < 0.05$				$P < 0.01$				$P < 0.01$			

well developed and considerably larger (mean fork length = 33.9 mm, N = 30) than is usual for Arctic char at emergence. McCart et al. (1972) record a mean fork length at emergence of 23.7 mm (range 20–27 mm) for the fry of anadromous Arctic char in tributaries to the Sagavanirktok River, Alaska. It appears that the fry from Cache Creek Spring had already been feeding for some time prior to May 10. By July 4, 1973 they had attained a mean length of 47.4 mm (N = 30).

Ages were determined from the otoliths of a sample of 70 fish (Table 4). The sample includes fish taken November 11, 1972 (N = 12), April 19, 1973 (N = 50), and May 10, 1973 (N = 8). The autumn sample was taken after growth had ceased and the two spring samples before any new growth was indicated on the otoliths. Ages were assigned to indicate length at annulus formation (i.e. at the end of each growth period) and therefore approximate calculated age–length data.

The growth of char in Cache Creek Spring exceeds that of fish inhabiting isolated springs in the Canning River Drainage, Alaska (Fig. 2). The relationships for the Alaskan populations are based on data (McCart and Craig 1973) for samples taken between August 29 and September 14, 1972, toward the end

TABLE 4. Relationship of age to sex ratios, maturity and length of Arctic char captured above falls at Cache Creek Spring, Yukon Territory.

	Males		Females				Length	
Age	N	% mature	N	% mature	Total	% Male	Mean	SD
2	8	62	8	0	16	50	102.9	9.1
3	4	100	1	0	5	80	132.2	10.8
4	4	100	5	40	9	44	162.1	8.2
5	5	80	4	25	9	55	170.8	36.6
6	6	100	9	100	15	40	206.1	14.2
7	3	100	6	100	9	33	223.9	16.2
8	2	100	2	100	4	50	250.3	19.5
9			1	100	1	0	237	
10			2	100	2	0	241.0	3.0
Totals	32	87	38	60	70	46	171.9	52.1

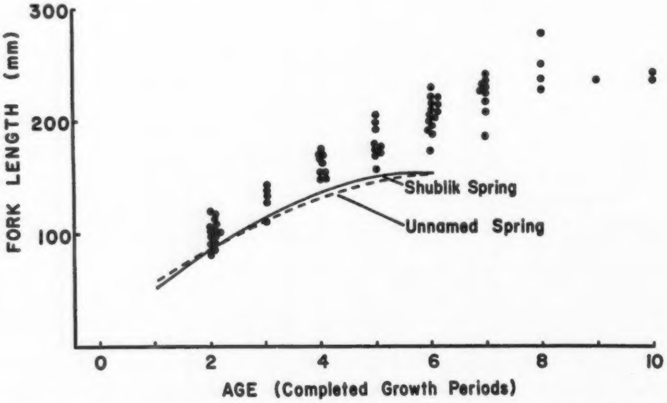


FIG. 2. Comparison of age–length relationship of Arctic char (*Salvelinus alpinus*) inhabiting isolated springs. Circles indicate data for individual fish from Cache Creek Spring. Data for Shublik Spring and Unnamed Spring, both in the Canning River Drainage, Alaska, are from McCart and Craig 1973.

of the growth period for both Canning populations. Thus, they also approximate calculated age–length relationships.

The Cache Creek Spring population may be longer-lived than either Canning River population. McCart and Craig (1973) report maximum ages of 8 for Shublik Spring (N = 165) and 7 for the unnamed Canning Spring (N = 141) compared with 10 for the Cache Creek Spring population (N = 70).

Males mature earlier than females and, with a single 5-yr-old exception, all males in the sample were mature by age 3. The youngest mature females were age 4 and they did not attain 100% maturity until age 6. It appears that once mature, fish of both sexes tend to spawn every year. In comparison with the Canning River Springs, males in Cache Creek Spring reach 100% maturity at an earlier, and females at a later age.

Discussion — The population of Arctic char inhabiting Cache Creek Spring upstream of the falls has had to adapt to an unusual environment. Because of the warm springs, there is a reversal of the normal temperature regime for streams in this area. Temperatures in the stream are highest during winter when the only sources of flow are the warm springs, and lowest during spring and summer when surface runoff contributes to stream flow. These high water temperatures are associated with low oxygen levels. Again, this is a reversal of the usual pattern for streams in the western Arctic in which low oxygen levels normally occur at low temperatures during winter when the long period of ice cover inhibits reoxygenation.

The Cache Creek Spring Arctic char population is of interest because it demonstrates that in some circumstances char can adapt to what appears to be an extremely unfavorable low-oxygen, high-temperature, high-salinity environment. Holeton (1973) demonstrated that at 2 C, a temperature within the normal range for overwintering fish, Arctic char from a high arctic lake exhibited a very low oxygen uptake (log O_2 uptake = 0.7316 log weight −1.0944). There was no evidence of cold adaptation and uptake at 2 C was comparable to projections of oxygen uptake vs. temperature relations for salmonid fish from more temperate latitudes. Holeton found that even at 2 C, his char showed signs of asphyxia and loss of equilibrium at an oxygen tension of 25 mm Hg (2.75 mg O_2 per liter). This oxygen concentration is higher than those recorded in the mainstream in the vicinity of the major orifice of Cache Creek Spring (Table 2), where normally behaving fish were observed in water of a much higher (15–16 C) temperature. However, Holeton's fish were completely acclimated to high ambient oxygen levels and then abruptly subjected to low oxygen concentrations. Were the fish acclimated to low oxygen levels or subjected to a more gradual reduction in oxygen levels, improved resistance to low oxygen levels might be expected. Shepard (1955) demonstrated improved tolerance to low oxygen levels in acclimated individuals of a related species, *Salvelinus fontinalis*.

A combination of high water temperature and low oxygen concentration could be expected to subject the char population in Cache Creek to a considerable metabolic stress. It is likely that fish can and do avoid the most stringent conditions by distributing themselves well downstream of the major orifices and by seeking out locally favorable areas. However, the presence of fish in the immediate vicinity of the major sources of warm, low-oxygen water and the high incidence of gas bubble disease suggest that many fish do inhabit marginal habitats during the winter. Despite this, the population appears to be thriving. Growth rates and longevity both exceed those of the two isolated populations in the Canning River Drainage (McCart and Craig 1973) which occupy seemingly more typical habitats.

We cannot, on the basis of the available evidence, determine whether the Cache Creek Spring fish avoid asphyxia by seeking out locally favorable areas or whether there is some physiological adaptation to conditions in the spring (i.e. reduced metabolism at high temperatures). If the latter, it would be interesting to know whether the adaptation has been selected for within the Cache Creek Spring population or is a result of acclimation and therefore a characteristic of the species as a whole. A comparison of the respiratory metabolism of the Cache Creek Spring population with that of Arctic char from other areas would be most interesting.

The meristic data (Table 3) indicate that the Arctic char upstream and downstream of the falls on Cache Creek constitute two distinct populations. However, the extent to which the differences are genetically as opposed to environmentally controlled is not known. Meristic characters can be modified by various environmental factors including water temperature and oxygen concentrations (Tåning 1952) and salinity (Heuts 1949). It is likely that each of these factors differs upstream and downstream of the falls.

Northcote et al. (1970) compared rainbow trout (*Salmo gairdneri*) from populations above and below an impassable falls and found significant differences in first-arch gillraker counts, vertebral numbers and number of parr marks. Interestingly the differences were in the same direction as those reported here (gillraker and parr mark numbers were greater above the falls and vertebral counts lower). However, we note that in this instance, there were no marked differences in environmental conditions above and below the falls. We suggest the meristic differences may be due to the fact that water temperatures during incubation differ because fish above the falls spawn later in the spring (presumably at higher temperatures) than those below.

Acknowledgments — This study was funded in part by a grant from the National Research Council of Canada and in part by the Canadian Arctic Gas Study Limited, Calgary, Alta.

Davies, H. S. 1965. Culture and diseases of game fishes. Univ. California Press, Berkeley, 332 p.

Holeton, G. F. 1973. Respiration of Arctic char (*Salvelinus alpinus*) from a high arctic lake. J. Fish. Res. Board Can. 30: 717–723.

Huets, M. J. 1949. Racial divergence in fin ray variation patterns in *Gasterosteus aculeatus*. J. Genet. 49: 183–191.

McCart, P., and P. Craig. 1971. Meristic differences between anadromous and freshwater-resident Arctic char (*Salvelinus alpinus*) in the Sagavanirktok River Drainage, Alaska. J. Fish. Res. Board Can. 28: 115–118.

1973. Life history of two isolated populations of Arctic char (*Salvelinus alpinus*) in spring-fed tributaries of the Canning River, Alaska. J. Fish. Res. Board Can. 30: 1215–1220.

McCart, P., P. Craig, and H. Bain. 1972. Report on fisheries investigations in the Sagavanirktok River and neighboring drainages. Report to Alyeska Pipeline Service Company, Bellevue, Washington. 125 p.

McPhail, J. D. 1961. A systematic study of the *Salvelinus alpinus* complex in North America. J. Fish. Res. Board Can. 18: 793–816.

Northcote, T. G., S. N. Williscroft, and H. Tsuyuki. 1970. Meristic and lactate dehydrogenase genotype differences in stream populations of rainbow trout below and above a waterfall. J. Fish. Res. Board Can. 27: 1987–1995.

Shepard, M. P. 1955. Resistance and tolerance of young speckled trout (*Salvelinus fontinalis*) to oxygen lack, with special reference to low oxygen acclimation. J. Fish. Res. Board Can. 12: 387–446.

Tåning, Å. V. 1952. Experimental study of meristic characters in fishes. Biol. Rev. 27: 169–193.

van Everdingen, R. O. 1972. Thermal and mineral springs in the southern Rocky Mountains of Canada. Environment Canada, Water Management Service, Ottawa. 151 p.

1973a. Analytical results for water samples collected by Northern Engineering Services, Calgary. I. Beaufort Sea Drainage, Yukon Territory, Canada. Water Resources Branch, Inland Waters Directorate, Calgary, Alta. 19 p. (unpublished report)

1973b. Analytical results for water samples collected by Aquatic Environments Limited, Calgary. I. Beaufort Sea Drainage, Yukon Territory, Canada. Hydrology Research Division, Water Resources Branch, Environment Canada. 13 p. (unpublished report)

Growth and Survival of Haddock (*Melanogrammus aeglefinus*) Larvae in Relation to Planktonic Prey Concentration

GEOFFREY C. LAURENCE

National Marine Fisheries Service, Northeast Fisheries Center
Narragansett Laboratory, Narragansett, R.I. 02882, USA

LAURENCE, G. C. 1974. Growth and survival of haddock (*Melanogrammus aeglefinus*) larvae in relation to planktonic prey concentration. J. Fish. Res. Board Can. 31: 1415–1419.

When newly hatched haddock (*Melanogrammus aeglefinus*) larvae were fed zooplankters at nominal rates of 0.5, 1.0, and 3.0/ml in the laboratory at 7 C, they grew at similar rates. After 6 wk, they averaged 8.7, 10.0, and 11.2 mm in standard length and 810, 1300, and 1728 μg in dry weight, and had condition factors of 1.25, 1.22, and 1.32. When fed at 0.1 and 0.01 plankters/ml, all larvae died in 3 and 2 wk; at 0.5–3.0 plankters/ml, daily instantaneous mortality coefficients were 0.06–0.02 during 6 wk. Larvae began feeding 2 days after hatching, and the point of no return after they were deprived of food was 6 days after hatching, yolk absorption being completed on day 6 or 7. All larvae deprived of food until 8 and 10 days after hatching, although initiating feeding, did not survive another 4 days. Prey concentration also influenced delayed feeding with greater percentages of larvae able to initiate feeding at higher plankton levels on the point-of-no-return day.

LAURENCE, G. C. 1974. Growth and survival of haddock (*Melanogrammus aeglefinus*) larvae in relation to planktonic prey concentration. J. Fish. Res. Board Can. 31: 1415–1419.

Les larves d'aiglefin (*Melanogrammus aeglefinus*) nouvellement écloses croissent au même rythme, lorsque nourries de zooplankton à des taux nominaux de 0.5, 1.0 et 3.0 organismes/ml en laboratoire à 7 C. Au bout de 6 semaines, elles ont en moyenne une longueur standard de 8.7, 10.0 et 11.2 mm, un poids sec de 810, 1300 et 1728 μg, et un indice d'embonpoint de 1.25, 1.22 et 1.32. Nourries à des taux de 0.1 et 0.01 organismes/ml, toutes les larves meurent au bout de 3 et 2 semaines; à des taux de 0.5–3.0 organismes/ml, les coefficients de mortalité instantanée sont de 0.06–0.02 pendant 6 semaines. Les larves commencent à se nourrir 2 jours après éclosion, et le point critique, sans retour possible, lorsqu'elles sont privées de nourriture, se situe à 6 jours après éclosion, la résorption du vitellus étant complète au jour 6 ou 7. Toutes les larves privées de nourriture jusqu'à 8 et 10 jours après éclosion, bien qu'elles commencent à se nourrir, meurent après 4 jours additionnels. La concentration des proies affecte également l'alimentation différée, un plus fort pourcentage de larves étant capables de commencer à se nourrir au jour critique lorsque les concentrations de plancton sont plus élevées.

Received February 11, 1974
Accepted May 15, 1974

Reçu le 11 février, 1974
Accepté le 15 mai, 1974

IT has been demonstrated for haddock (*Melanogrammus aeglefinus*) that a large differential mortality occurs during the planktonic larval stage which may affect the recruit level of a year-class (Parrish 1950; Saville 1956). Likely causes of large differential mortalities between years are the availability of a suitable food supply in sufficient quantity and the critical timing involved between prey encounter and feeding initiation. This research was designed to examine the influence of five nominal planktonic prey concentrations (3.0, 1.0, 0.5, 0.1, and 0.01 plankters/ml) on growth, survival, and condition factor during the first 6 wk after hatching, and the time larvae could survive in the absence of food and still retain the ability to initiate successful feeding (point of no return).

Materials and methods — Fertilized eggs were obtained from a pair of naturally spawning adults in the 56,700-liter experimental aquarium of the Narragansett Laboratory. They were collected with a ½ m plankton net shortly after spawning. After collection, 1000 eggs (approximated volumetrically) were placed in sea water (29‰) in each of five 37.8-liter all glass aquaria painted black for the prey concentration studies. For the point-of-no-return studies, 100 newly hatched larvae were

Printed in Canada (J3312)
Imprimé au Canada (J3312)

placed in 3.8-liter plastic aquaria painted black. All experimental aquaria were provided with airstones, and the temperature was maintained at 7 ± 0.5 C. Photoperiod was manipulated to approximate the normal day:night ratio during the haddock spawning season.

Zooplankton concentrations (Fig. 1) were established at hatching by maintaining a constant volume of sea water in each aquarium and adding the desired number of plankters. Counts for the concentrations were based on the mean of duplicate 5-ml samples from each aquarium after gentle stirring. Concentrations were maintained generally near the nominal values by adding, every day or every second day, known quantities of zooplankton taken with 35-μ mesh plankton nets from the Narragansett Bay area and sieved through 210-μ mesh strainers. The addition of sea water with zooplankton caused the salinity to vary between 28 and 30‰ during the experiment.

The zooplankters were mainly nauplii of *Acartia clausi*, *Centropages hamatus*, and *Balanus balanoides* and included some adults of *A. Clausi* and *Podon* sp., all known as food items of larval haddock in field studies (Marak 1960; Ogilvie 1938).

As all eggs and larvae came from the same spawning, initial dry weights and standard lengths were measured on the basis of a sample of 10 larvae (two from each of the five experimental aquaria) taken before food was introduced. Thereafter, 10 larvae from each of the experimental concentrations were collected every 7 days for measurements. Dry weight was determined by rinsing larvae in distilled water, pipetting onto a glass petri dish, drying to a constant weight at 90 C for 24 h, and weighing individually to the nearest 0.1 μg on a Chan Gram electrobalance. Standard lengths were measured to the nearest 0.1 mm with a filar ocular micrometer in a dissecting microscope.

Condition factors were measured weekly after yolk was absorbed and were calculated by dividing the mean dry weight (μg) by the cube of the mean standard length (mm).

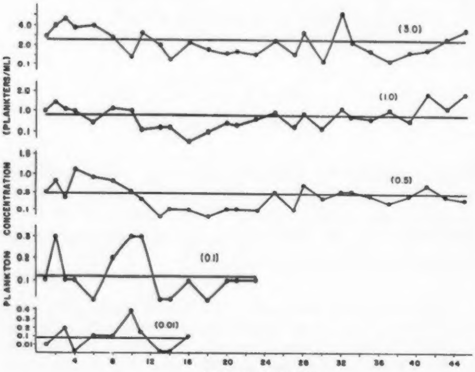


FIG. 1. Monitored plankton concentrations for the five nominal concentrations (in parentheses) for the duration of the experiment. Horizontal lines represent mean monitored values.

Average daily instantaneous mortality coefficients were calculated from the formula:

$$Z = \frac{\log_e S_t - \log_e S_{t'}}{T} \qquad (1)$$

where S_t and $S_{t'}$ are the numbers of survivors at the beginning and end of the time interval in days (T). Numbers surviving at the beginning of T were calculated from studies of G. C. Laurence and C. A. Rogers (unpublished data) which indicated that 353 of 1000 fertilized haddock eggs hatched when subjected to 7 C and 28‰, and numbers surviving to the end of T were determined by actual count. To correct S_t for potential survivors that had been removed in the sampling, the following formula was used:

$$\hat{S}_{t'} = S_{t'} + 10 \sum_{k=1}^{w-1} e^{-7KZ} \qquad (2)$$

where 10 = number of individuals removed each week, w = number of weeks of sampling, 7 = days in 1 wk, Z = average daily instantaneous mortality coefficient.

The initial Z value in equation (2) was chosen to reflect the maximum possible mortality rate, i.e. no survivors from the samples taken, and was used to iteratively correct $S_{t'}$ in equation (1) so as to give an unbiased estimate of Z for each experiment.

Daily specific growth rates were calculated from the formula:

$$\text{SGR} = 100 \frac{\text{Log}_e \text{GT} - \text{Log}_e \text{G}_t}{\text{T}}$$

where GT and G_t are the sizes (mean dry weight) at the beginning and end of the time interval in days (T).

The point-of-no-return experiment was designed to determine the latest point after hatching when food had to become available for the larvae to initiate successful feeding. Larvae in separate aquaria were fed ad libitum (approximately 2 plankters/ml) starting at successive 2-day intervals from hatching, and samples of 10 larvae were taken every 24 h after feeding and analyzed for stomach contents to determine when 50% or more fed or it was obvious that larvae could not feed successfully. Then an experiment was set up at the 5 plankton concentrations in the feeding level studies to determine if plankton concentration on the day of the point of no return affected feeding capability.

All statistical analyses of the growth data used in these experiments are described in Steel and Torrie (1960).

Mortality — Daily instantaneous mortality coefficients for the duration of the experiments from hatching decreased with increasing feeding level at 3.0, 1.0, and 0.5 pl/ml. The total numbers of survivors and the unbiased coefficients for the three prey concentrations were:

Concn (pl/ml)	3.0	1.0	0.5
No. of survivors	139	79	11
Coefficient (z)	0.02	0.03	0.06

Mortality was 100% at 0.1 and 0.01 pl/ml in 2 and 3 wk.

When larvae were deprived of food until 8 and 10 days after hatching and then provided with plankters ad libitum, all died within another 4 days even though more than 50% had food in their alimentary tracts. Larvae deprived of food until day 12 after hatching were all dead on day 12 apparently of starvation. Yolk absorption was complete on day 6 or 7, and 50% or more of the larvae deprived until days 2, 4, and 6 all initiated feeding and remained healthy to day 14 when observations ceased. These results indicated that day 6 after hatching was the point of no return, and that the criterion of 50% feeding initiation was not necessarily valid for haddock larvae without subsequent observation.

The percentages of larvae that fed on day 6 at the 5 prey concentrations and subsequently survived were:

Concn	3.0	1.0	0.5	0.1	0.01
% feeding	80	60	50	30	10

Growth — On the basis of the semilogarithmic transformations of the growth in mean dry weight and mean standard length vs. weeks after hatching and the slopes of the regression equations (Fig. 2), growth at 0.5–3.0 pl/ml tended to increase ($P > 0.05$) with prey concentration. Of course, the total mortality at concentrations of 0.1 and 0.01 pl/ml negated any growth at these feeding levels.

Condition factors of larvae increased with time for all feeding levels (Fig. 2), the rates of increase being randomly associated with feeding level.

Discussion — Relating mortality estimates of these experiments to those determined in laboratory studies of other species reveals some similarities. O'Connell and Raymond (1970) working with northern anchovy (*Engraulis mordax*) larvae recorded high mortalities at concentrations of 0.1 copepod nauplius/ml and below, as in these studies. Saksena and Houde (1972) found variable survival of bay anchovy (*Anchoa mitchilli*) and scaled sardine (*Harengula pensacolae*) larvae in the laboratory, but concluded that prey concentrations of 0.5–1.0 copepod nauplius/ml provided adequate food for growth and survival.

The mortality percentages noted for prey concentrations of 0.1–3.0 pl/ml in this research are similar to field estimates noted by other researchers. Saville (1956) recorded field estimates of daily mortality percentages for haddock larvae from hatching to 10–21 mm in size, which approximates the sizes of haddock in this research. His estimates for 2 yr range from 4.8 to 10.7%. A calculation based on Colton's (1965) field data for Georges Bank larval haddock produced mortality rates of 6.5–7.7%/day. This may indicate that the present laboratory studies approximate conditions in the natural environment.

The fact that condition factor at each of the three higher feeding levels increased with time indicated a disproportionate increase in dry weight growth as compared with the cube of standard length growth. Blaxter (1965) also noted an increase in condition factor in healthy herring larvae and attributed it to the result of allometric growth and the addition of skeletal structures. The interpretation of condition factors is apt to be difficult; however, it does give a basis for comparison of the treatments (planktonic-prey concentrations) in these experiments.

The specific growth rates in standard length computed in these laboratory studies at 7 C from the data of Fig. 2 were 1.12, 0.99, and 0.85%/day at 3.0, 1.0, and 0.5 pl/ml. These rates, especially at 3.0 pl/ml, are similar to results of Saville (1956) who estimated larval haddock growth rates in terms of length from field data. His computed growth coefficient (no temperature data given) for 40–45 days after hatching was 0.0118, or a specific growth rate on a daily basis of 1.18%. These are also very similar to the growth rate of 1.15%/day calculated by Saville from Walford's (1938) field research. In view of the 100% mortality at concentrations of 0.1 and 0.01 pl/ml and the similarity of these laboratory growth rates to the computed field estimates, it appears that haddock larvae may need prey concentrations of 0.5–3.0 pl/ml for suitable growth.

The results of this laboratory research corroborate several field investigations in which prey concentrations encountered by larvae were directly related to their abundance or survival. Lisivnenko (1961) noted that in years when plankton organisms were less than 0.01 pl/ml larval Baltic herring were scarce in field collections, and in years when concentrations were higher than 0.01 pl/ml larvae were much more abundant. The same relationship with an apparent critical density of 0.01 pl/ml was recorded for the Black Sea anchovy (Pavlovskaya 1961). Sysoeva and Degtereva (1965) reported that the minimum abundance of *Calanus finmarchicus* in the plankton when the intensity of feeding of cod larvae decreased was from 0.01 to .005/ml, and that a concentration of 0.02/ml provided sufficient food for good survival. Bogorov (1939) discovered that cod larvae were much more abundantly associated with water masses where the number of copepod nauplii was 0.01/ml than at lower concentrations.

The above field study estimates of prey concentrations indicate larval success for some species at concentrations somewhat lower than indicated for haddock in this laboratory study. However, the field study prey concentrations represent average density over a linear or oblique distance sampled by a plankton net. Copepods have contagious distributions and larvae may well be associated with clumps of prey that are more densely concentrated than indicated in a plankton tow made over some arbitrary distance. The range of densities in this experiment may approximate at least a part of the range of densities encountered by larval haddock in contagiously distributed plankton.

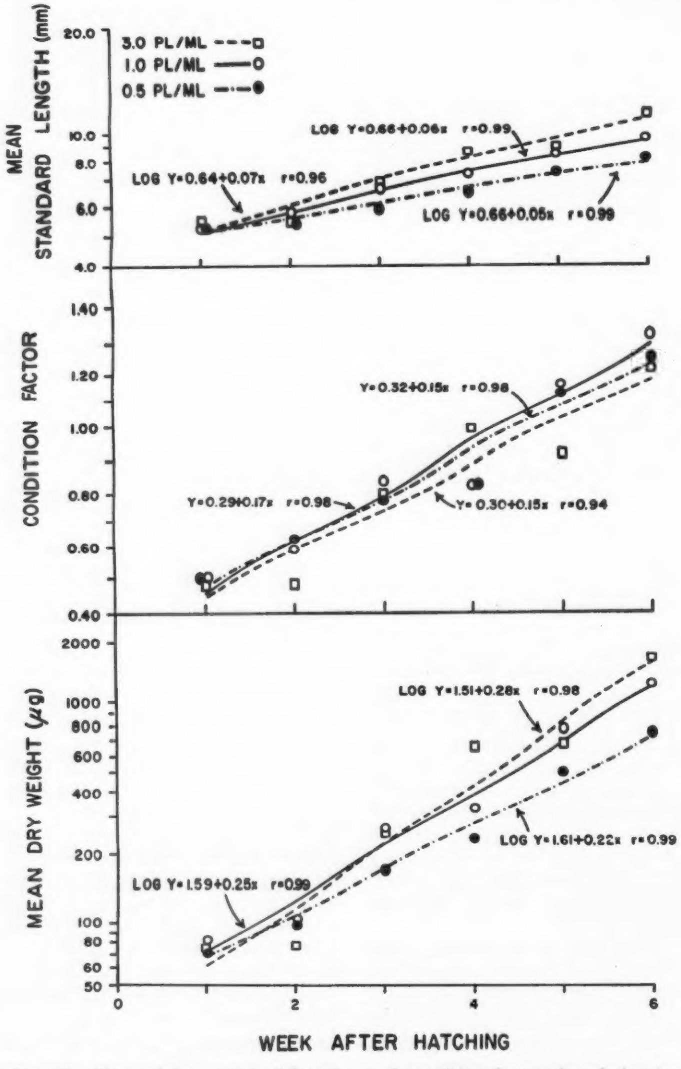


FIG. 2. Dry weights, standard lengths, and condition factors in relation to week after hatching of haddock larvae fed at three zooplankton concentrations at 7 C.

The delayed feeding study at 7 C revealed that the criterion of 50% of a sample of larvae initiating feeding after deprivation is not valid for establishing a point of no return for haddock and that prey concentration directly affects the ability of the larvae to initiate feeding. As far as is known results like these have not been demonstrated before with other species and show that the point of no return may be flexible. Ability to feed after deprivation depends on several other factors including species, initial egg size or yolk reserve, and temperature. The point after which larvae are living but unable to feed successfully has been reported to vary from as few as 4 days for bluegill larvae (*Lepomis macrochirus*) at 23.5 C (Toetz 1966) to 36 days at 3–4 C for cisco (*Leucichthys artedii*, John and Hasler 1956) with many intermediate values (Blaxter 1970). Some researchers have considered delayed feeding ability as a critical factor in relation to larval survival. Toetz (1966) defined the so-called "critical period" of larval survival as being from feeding initiation to the point of no return.

This laboratory research implies that haddock larvae do have a critical period in which mortality may be great, that it is a relatively short time during the first weeks after hatching, and that it is controlled to a certain degree by the critical timing of larvae encountering planktonic prey in sufficient numbers so that successful feeding is promoted.

Acknowledgments — I wish to acknowledge Dr D. Au, Mr J. B. Colton Jr., Mr R. C. Hennemuth, and Mr R. R. Marak for their critical review of the manuscript. Thanks also go to Mr T. Halavik and Mr A. Smigielski for their technical help, to Ms M. Marsh who drafted the figures, and to Mr Hennemuth and Dr E. Heyerdahl who aided in the calculation of the unbiased mortality coefficients.

BLAXTER, J. H. S. 1965. The feeding of herring larvae and their ecology in relation to feeding. Calif. Coop. Oceanic Fish. Invest. Vol. X: 79–88.

1970. Development: eggs and larvae, p. 178–241. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. II. Academic Press, Inc., New York, N.Y.

BOGOROV, V. G. 1939. Characteristics of seasonal appearances in the plankton of polar seas and their significances for forecasts of ice [Osobennosti sezonnykh yavlennii v planktone polyarnykh morei i ikh z nachenie dlya ledovykh prognozov]. Zool. Zh. Vol. 18(5): 17–32.

COLTON, J. B. JR. 1965. The distribution and behavior of pelagic and early demersal stages of haddock in relation to sampling techniques. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 6: 317–333.

JOHN, K. R., AND A. D. HASLER. 1956. Observations on some factors affecting the hatching of eggs and the survival of young shallow-water cisco, *Leuchichthys artedi*. Le Sueur, in Lake Mendota, Wisconsin. Limnol. Oceanogr. 1: 176–194.

LISIVNENKO, L. N. 1961. Plankton and feeding of larvae of the Baltic herring in the Riga Gulf. Tr. Nauchno-Issled. Inst. Ryb. Khas. Latvian SSR 3: 105–138.

MARAK, R. R. 1960. Food habits of larval cod, haddock, and coalfish in the Gulf of Maine and Georges Bank area. J. Cons. Int. Explor. Mer 25: 147–157.

O'CONNELL, C. P., AND L. P. RAYMOND. 1970. The effect of food density on survival and growth of early post yolk-sac larvae of the northern anchovy *Engraulis mordax* Girard) in the laboratory. J. Exp. Mar. Biol. Ecol. 5: 187–197.

OGILVIE, H. S. 1938. The food of post larval haddock with reference to the annual fluctuations in the haddock broods. Rapp. P-V Reun. Cons. Perm. Int. Explor. Mer. 107(3): 57–66.

PARRISH, B. B. 1950. Brood fluctuations in the North Sea haddock population. Challenger Soc. Abstr. 3(2): 18.

PAVOLVSKAYA, R. M. 1961. La survie des larves de l'anchois de la Mer Noire en fonction de leur alimentation. Rapp. Comm. Int. Mer Medit. XVI: 345–350.

SAKSENA, V. P., AND E. D. HOUDE. 1972. Effect of food level on the growth and survival of laboratory-reared larvae of bay anchovy (*Anchoa mitchilli* Valenciennes) and scaled sardine (*Harengula pensacolae* Goode and Bean). J. Exp. Mar. Biol. Ecol. 8: 249–258.

SAVILLE, A. 1956. Eggs and larvae of haddock (*Gadus aeglefinus* L.) at Faroe. Mar. Res. 1956(4): 1–27.

STEEL, R. G. D., AND J. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill Book Co., Inc., New York, N.Y. 481 p.

SYSOEVA, T. K., AND A. A. DEGTERVA. 1965. The relation between the feeding of cod larvae and pelagic fry and the distribution and abundance of their principle food organisms. Int. Comm. Northwest Atl. Fish. Spec. Publ. No. 6: 411–416.

TOETZ, D. W. 1966. The changes from endogenous to exogenous sources of energy in bluegill sunfish larvae. Invest. Indiana Lakes Streams 7(4): 115–146.

WALFORD, L. A. 1938. Effects of currents on distribution and survival of the eggs and larvae of the haddock (*Melanogrammus aeglefinus*) on Georges Bank. Bull. U.S. Bur. Fish. 49(29): 1–73.

Development of Steelhead Trout (*Salmo gairdneri*) Otoliths and Their Use for Age Analysis and for Separating Summer from Winter Races and Wild from Hatchery Stocks[1]

J. L. McKERN[2] AND H. F. HORTON

Department of Fisheries and Wildlife
Oregon State University, Corvallis, Oreg. 97331, USA

AND K V. KOSKI[3]

Fisheries Research Institute
University of Washington, Seattle, Wash., USA

McKERN, J. L., H. F. HORTON, AND K V. KOSKI. 1974. Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks. J. Fish. Res. Board Can. 31: 1420–1426.

In steelhead trout (*Salmo gairdneri*) the first pair of otoliths (sagittae) appeared in X-ray photographs of embryos 14 days before hatching and were the first calcified structures visible. Subsequent growth characteristics of otoliths were used in identifying as freshwater and ocean annuli and spawning checks, and indicated seven age-classes and 26 life history patterns among 434 specimens. Diameters of otolith nuclei were smaller ($P \leq 0.01$) in summer steelhead (mean = 0.348 mm) than in winter steelhead (mean = 0.436 mm), and diagonally measured otolith growth representing the first year of life was larger ($P \leq 0.01$) in hatchery-reared (1.291 mm) than in wild steelhead (0.868 mm for 2/ fish and 0.769 mm for 3/ fish). Absence of changes in density in the freshwater growth of otoliths from hatchery-reared steelhead and presence of such changes in the otoliths from wild steelhead served as a subjective means of accurately separating the two stocks.

McKERN, J. L., H. F. HORTON, AND K V. KOSKI. 1974. Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks. J. Fish. Res. Board Can. 31: 1420–1426.

Chez la truite steelhead (*Salmo gairdneri*), la première paire d'otolithes (sagittae) apparaît sur des radiographies d'embryons 14 jours avant l'éclosion. Ce sont les premières structures calcifiées à apparaître. Les caractéristiques de la croissance subséquente des otolithes ont servi à identifier les annuli formés en eau douce et en mer, ainsi que les marques annulaires supplémentaires de fraie, et ont révélé la présence de sept classes d'âge et 26 modalités de cycle biologique parmi 434 spécimens. Le diamètre du noyau de l'otolithe est plus petit ($P \leq 0.01$) chez la truite steelhead d'été (moyenne = 0.348 mm) que chez celle d'hiver (moyenne = 0.436 mm). La croissance de l'otolithe représentant la première année de vie, mesurée en diagonale, est plus grande ($P \leq 0.01$) chez la truite steelhead de pisciculture (1.291 mm) que chez la truite sauvage (0.868 mm pour des poissons de 2 ans et 0.769 mm pour des poissons de 3 ans). L'absence de changements de densité dans la croissance d'eau douce des otolithes chez les truites steelhead de pisciculture et la présence de tels changements sur les otolithes de truites sauvages ont fourni un moyen subjectif de séparer avec justesse les deux stocks.

Received August 13, 1974
Accepted May 13, 1974

Reçu le 13 août, 1974
Accepté le 13 mai, 1974

---

[1] Technical Paper No. 3138, Oregon Agricultural Experiment Station.

[2] Present address: U.S. Army Corps of Engineers, Walla Walla District, Walla Walla, Wash. 99362, USA.

[3] Present address: Forest Sciences Laboratory, Box 909, Juneau, Alaska 99801, USA.

Printed in Canada (J2273)
Imprimé au Canada (J2273)

THIS report is on development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and spawning history and for separating summer from winter races[4] and wild from hatchery-reared stocks.[5] Because anadromous salmonids tend to resorb scale margins during spawning migrations and to regenerate lost scales, some authors (McMurrich 1910, 1913; Clutter and Whitesel 1956; Kim 1963; Bilton and Jenkinson 1968; Kim and Roberson 1968) use otoliths rather than scales for life history studies and for stock identification of certain Pacific salmon (*Oncorhynchus*). Except for a preliminary study on 18 fish by K V. Koski (unpublished data), the use of steelhead otoliths for these purposes has not been investigated. The present study was conducted from 1968 to 1970 on steelhead from 10 rivers in the Pacific northwest; details of the study, including appendices of raw data, were presented by McKern (1971).

Materials and methods — Otoliths, scales, and pertinent data were obtained from steelhead of rivers in Oregon, Washington, and British Columbia (Table 1). Developing embryos, fry, and juveniles of Alsea River winter steelhead were obtained from hatchery-reared stocks furnished by the Oregon Wildlife Commission. Samples and data collected usually consisted of otoliths, scales, location of capture, origin (wild or hatchery), hatchery marks if present, fork length, sex, and date of capture. Otoliths of 90 embryos and fry, which were preserved in buffered 10% Formalin and stored 1–3 wk in 36% isopropyl alcohol, were studied in negatives of X-ray photographs. Otoliths were obtained from advanced fry by splitting their heads under a dissecting microscope and probing for the small, granular sagittae. Otoliths of juveniles and adults were collected as described by McKern and Horton (1970) except that they were obtained by splitt-

---

[4] A number of stocks grouped together on the basis of similarity of migration times, and synonymous with "run" as defined by Ricker (1972).

[5] The salmon of one species which form rather clear-cut breeding populations (Larkin 1972).

TABLE 1. Mean nucleus diameters of otoliths from summer and winter races of wild and hatchery-reared steelhead trout (*Salmo gairdneri*) from rivers in Oregon, Washington, and British Columbia (1968–70).

River	Sample size	Nucleus diam (*mm*) Mean ± 1 SD
	Summer	
Rogue R., Oreg., hatchery	2	0.367±0.014
wild	4	0.369±0.012
Siletz R., Oreg., hatchery	24	0.359±0.022
wild	5	0.346±0.021
Subtotal	29	0.354±0.022[a]
Washougal R., Wash., hatchery	53	0.343±0.027
Chambers Ck., Wash., hatchery[c]	97	0.346±0.024
Total	214	0.348±0.024[b]
	Winter	
Alsea R., Oreg., hatchery	18	0.429±0.031
wild	7	0.431±0.027
Siletz R., Oreg., hatchery	18	0.430±0.026
wild	20	0.432±0.027
Subtotal	38	0.431±0.026[a]
Skagit R., Wash., hatchery	56	0.449±0.025
Hoh R., Wash., wild	12	0.440±0.027
Big Beef Ck., Wash., wild	16	0.422±0.011
Nanaimo R., B.C., wild	10	0.429±0.033
Big Qualicum R., B.C., wild	18	0.459±0.022
Total	213	0.436±0.033[b]

[a,a;b,b] Each pair of means significantly different at $P \leq 0.01$ (Student's t test).

[c] Of Washougal River origin, but reared in Chambers Creek.

ing the heads of the fish on the few occasions on which only the frozen heads were available. The first three scales above the lateral line in the diagonal row extending from the front of the dorsal fin posteroventrally to the lateral line were collected from each side of the fish except when only the frozen heads of fish were available.

Otoliths were stored dry either in plastic concavity plates (Kim 1963) or in coin envelopes, or retained in the frozen heads of fish. Prior to examination, otoliths were transferred to liquid-filled vials for a minimum clearing time of 2 h in tap water or in 50:50 glycerin and water. No deterioration was noted in the readability of otoliths stored as long as 6 mo in these two media. Scales were mounted on gummed tape, impressed on acetate cards, and read on a scale projector as described by Koo (1955).

Otoliths were examined under a binocular microscope utilizing light reflected from a black background as described by Kim (1963). To facilitate measurements, each otolith was photographed on direct positive film (ASA 80) and the frames were mounted in 35-mm slides. The magnification used for all photographs was 10×, and the slides were projected for measurements such that the image of each otolith was magnified 100×.

For assigning ages from otoliths, winter growth in fresh water was taken to be represented by an uninterrupted, oval, hyaline zone (annulus) and winter growth in the ocean by a hyaline zone (annulus) interrupted by notches in the anterior and posterior regions of the otolith. The general terminology used to describe characteristics of otoliths was adapted from Kim and Koo (1963).

Otolith length was measured as the distance (millimeters) between perpendicular lines from the extremities of the postrostrum and the anterostrum (Fig. 1A). The first year of growth was measured along the diagonal axis from the center of the nucleus to the first annulus, and the 2nd yr as the distance from the center of the nucleus to the second annulus minus the 1st yr of growth (Fig. 1B). Each succeeding year of growth and total freshwater growth were similarly measured. "Plus-growth" (a narrow opaque zone of freshwater growth formed after the last freshwater annulus) equaled total freshwater growth minus measurement to the last freshwater annulus. The diameter of the nucleus was measured as the distance between the dorsal and ventral limits of the nuclear check (Fig. 1C).

Age was designated by the numbers of freshwater and ocean annuli, separated by a slash. For example, a 2/2 age steelhead (Fig. 1A) would have spent two winters in freshwater and two winters in the ocean. Completed spawning migrations, identified by the presence of spawning checks, were designated by "s." These spawning checks appeared as a cutting-back of one annulus into another in the ventral lobe of the otolith (Fig. 1D). Thus, a steelhead of age 2/2sls (Fig. 2) would have spent two winters in freshwater, two winters in the ocean followed by a spawning migration, and a third winter in the ocean followed by a spawning migration. Analogous features on scales (Fig. 2) corroborated our interpretations.

Comparison of age and spawning interpretations from otoliths with those from scales of 33 marked (hence, known age), hatchery-reared steelhead revealed that the otolith method was at least as accurate as the scale method. Four pairs of otoliths were unusable because of breakage or eroded margins, and scales from nine fish were unusable because of regenerated freshwater growth. Ages assigned to 27 of 29 otoliths (93%) and 21 of 24 scales (88%) agreed with known ages. For those fish whose ages were incorrectly assigned by either method, disagreement was associated with the number of ocean annuli or spawning checks.

Among 440 wild and hatchery-reared steelhead whose ages were not known, at least one otolith from each was readable in 405 cases (92%). Scales were available from 283 of these fish and assignment of age was possible in 231 cases (82%). Both otoliths from 35 fish and all scales from 52 fish were unusable for reasons stated above. Ages assigned for 200 fish using otoliths and scales independently were in agreement 186 times (93%).

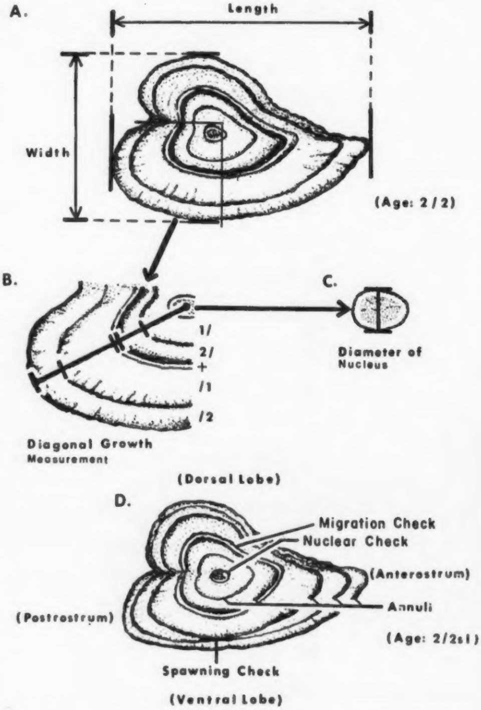


FIG. 1. Measurements and growth features used to describe the otoliths from steelhead trout (*Salmo gairdneri*).

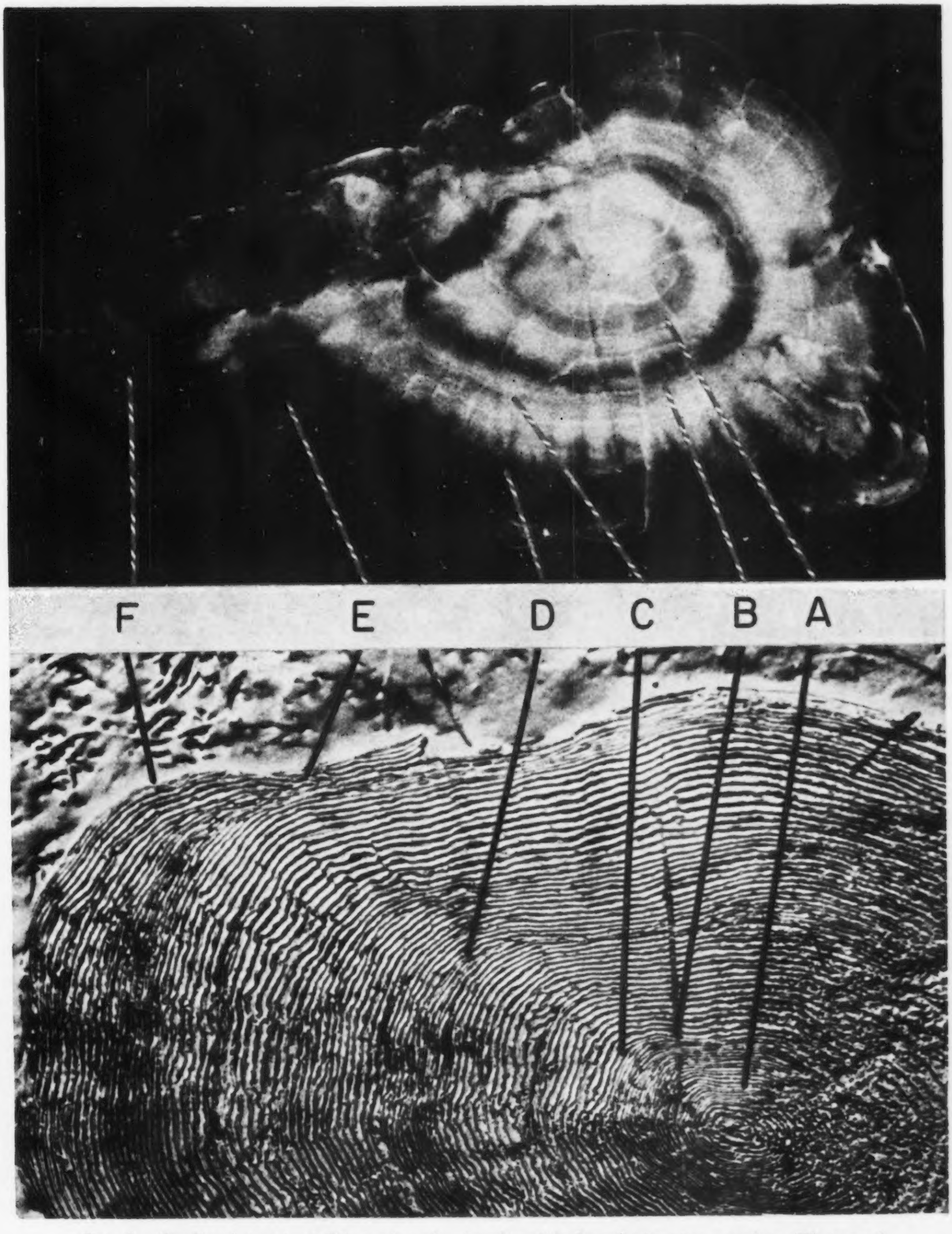


FIG. 2. Steelhead trout otolith and scale showing (A) first freshwater annulus, (B) second freshwater annulus, (C) migration check, (D) first saltwater annulus, (E) spawning check after 2 yr in the ocean, and (F) spawning check after 3 yr in the ocean (20×).

MecKern et al. — J. Fish. Res. Board Can.

Results — Development of otoliths — Otoliths were the first calcified structures identified in steelhead embryos, the first pair (Fig. 3A) being visible 14 days before the larvae (incubated at 12 C) hatched. Comparison of X-ray films of embryos with those of juveniles (Fig. 3E) verified that these were the sagittae. A second pair (lapilli) appeared 9 days before hatching (Fig. 3B), and the two pairs were the only calcified structures evident in the heads of embryos at hatching (Fig. 3C). At the time of complete absorption of the yolk, the sagittae had a hyaline nucleus surrounded by a narrow ring of opaque growth (Fig. 3D). The third pair of otoliths (asterisci) was not visible 5 days after hatching when the last X-ray photographs of fry were made but they were clearly visible in the heads of juveniles (Fig. 3E).

Six patterns of annual growth in fresh water developed on otoliths of juveniles, two for hatchery-reared and four for wild steelhead. In otoliths of hatchery-reared steelhead, zones of annual growth in fresh water were void of patterns (changes in density) in nearly all cases, indicating that migration to the ocean occurred immediately after liberation (at age 1 yr). In a few cases, however, occurrence of seasonal patterns in freshwater growth indicated at least 1 additional year's residence in fresh water following liberation. In otoliths of wild fish, seasonal patterns were evident in freshwater growth of each year of the 1–4 spent in fresh water. Plus-growth was not evident on otoliths of hatchery-reared steelhead, though it was found on otoliths of many wild fish. A migration check (a narrow hyaline zone delineating total freshwater growth, Fig. 1D) developed on otoliths from fish of each stock.

Seasonal patterns were found for ocean growth in otoliths of both stocks, and steelhead of each stock apparently spent 1, 2, or 3 yr in the ocean prior to their first spawning migration. Migration checks (Fig. 1D) indicated that fish of each stock spent 1 or 2 yr in the ocean between successive migrations.

Otolith length was in direct proportion to body (fork) length for steelhead of each race and stock, and for repeat spawners. Formulae for the regression of otolith length (Y) on fork length (X) of smolt and adult steelhead and the regression coefficients (r) are:

Wild winter steelhead:

$$Y = 2.15901 + 0.00564\ (X)\ (r = .969)$$

Hatchery winter steelhead:

$$Y = 1.77465 + 0.00589\ (X)\ (r = .980)$$

Hatchery summer steelhead:

$$Y = 2.22378 + 0.00559\ (X)\ (r = .967)$$

Repeat spawning steelhead:

$$Y = 2.18778 + 0.00572\ (X)\ (r = .815)$$

Differences were noted between the lengths of left and right otoliths from some individuals, and female steelhead tended to be larger than males in most of the rivers represented, but Student's t tests (Bruning and Kintz 1968) indicated that these variations had no significant effect ($P \leq 0.05$) on the relationship of otolith length to fork length in the total sample or in individual river samples.

Life history interpretations — Growth patterns on otoliths indicated that interfaces between winter and summer growth formed in late March or early April. Because steelhead spawning peaks in March, these interfaces represent the completion of a year in the life of the fish.

On the basis of the numbers of annuli, 434 fish were assigned to seven age-classes. The patterns concerning number of years in fresh (1–4) and in salt (1–5) water and frequency of spawning (1–2) totaled 26 out of a possible 104. About 78% of the fish were in the patterns 1/, 1/2, 2/2, and 3/2.

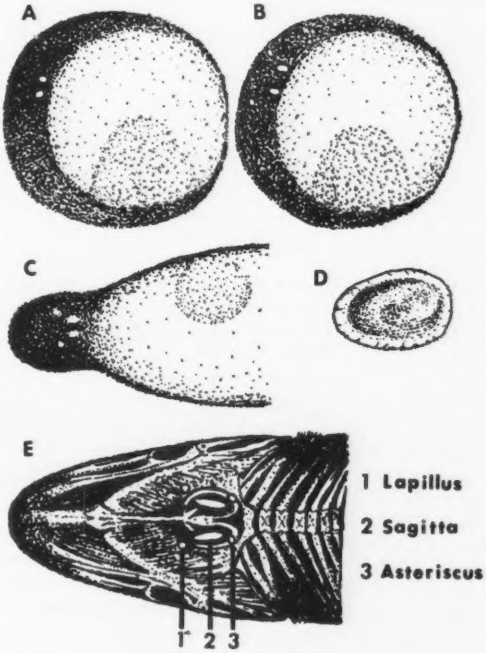


FIG. 3. Development of otoliths in steelhead trout as reproduced from X-ray films of (A) an ovum 14 days before hatching, (B) an ovum 9 days before hatching, (C) a newly hatched fry, (D) an otolith from a fry that had absorbed the yolk, and (E) a juvenile showing three pairs of otoliths.

The various patterns were similar to those determined by others from scale analysis (Maher and Larkin 1954; Shapovalov and Taft 1954; Withler 1965).

Separating summer and winter races — Size of otoliths provided a means for separating summer from winter races of wild and hatchery-reared steelhead. The mean nucleus diameter of otoliths of winter races were consistently larger ($P \leq 0.01$; Student's t test) than those of summer races (Table 1). The grand mean nucleus diameter (0.436 mm) of the winter races was also larger than that of the summer races (0.348 mm). A frequency diagram revealed only 5.7% overlap in a sample of 348 fish, and no overlap between Siletz River summer and winter steelhead (Fig. 4).

Separating wild and hatchery-reared stocks — Freshwater growth of otoliths in the first year was larger ($P \leq 0.01$; Duncan's t test (Bruning and Kintz 1968)) for hatchery-reared (1.291 mm) than for wild (0.868 mm for 2/fish and 0.769 for 3/ fish) steelhead (Table 2). There was no difference ($P \leq 0.05$) in total freshwater growth of otoliths between hatchery-reared (1.291 mm) and wild 2/ (1.328 mm) steelhead, even though differences existed ($P \leq 0.01$) between hatchery-reared (1.291 mm) and wild 3/ (1.440 mm) fish.

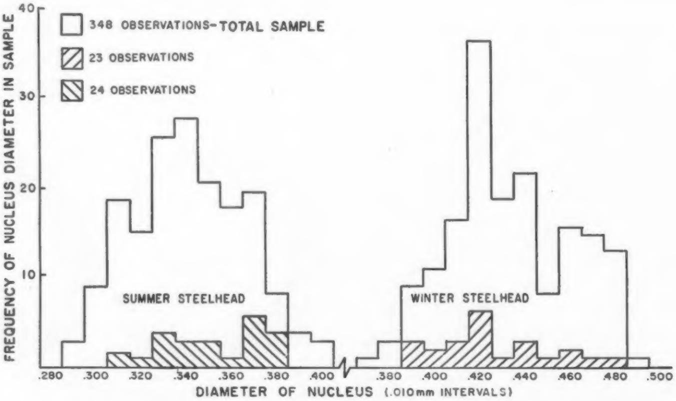


FIG. 4. Frequency distributions of diameters of otolith nuclei from summer and winter steelhead from the Siletz River, Oreg. (latched bars), and from all rivers studied in Oregon, Washington, and British Columbia (open bars) during 1968–70.

TABLE 2. Mean annual freshwater growth (mm ± SD; measured diagonally) of otoliths from wild and hatchery-reared steelhead trout of the streams studied, 1968–70.

Growth period	Hatchery	Wild	
	age 1/	age 2/	age 3/
Year 1	$1.291 \pm 0.099^{a,b}$	0.868 ± 0.079^{a}	0.769 ± 0.085^{b}
Year 2		$1.263 \pm 0.064^{c,d}$	1.092 ± 0.128^{c}
Year 3			1.377 ± 0.087^{d}
Plus-growth		0.066 ± 0.035	0.059 ± 0.053
Total	1.291 ± 0.099^{e}	1.328 ± 0.067^{f}	$1.440 \pm 0.074^{e,f}$
	(N = 315)	(N = 65)	(N = 41)

[a–f] Values with the same superscript differ (Duncan's t test) at $P \leq 0.01$ (a–c, e) or $P \leq 0.05$ (d,f).

The lack of seasonal patterns in freshwater growth of otoliths of hatchery-reared fish compared with the presence of such patterns in otoliths of wild fish (Fig. 5) provided an additional means to separate the two stocks. On the basis of the 186 specimens assigned identical ages by the otolith and scale methods, 99% of the hatchery fish and 98% of the wild fish conformed to the patterns indicated in Fig. 5.

Discussion — Comparison of the three most common patterns of growth in fresh water (Fig. 5) indicated that hatchery fish grew faster than wild fish, and 2-yr wild fish grew faster than those spending 3-yr in fresh water. Similar results were reported by Andrews (1959) for Alsea River steelhead.

Resorption due to spawning has not been reported previously concerning otoliths. Rollefsen (1935) described spawning checks as part of the pattern of continuous growth of cod (*Gadus morhua*) otoliths; but in steelhead, spawning checks resulted from what appeared to be resorption of the ventral edge of the otolith (the edge used in aging). This apparent resorption of the ventral edge also has been observed by H. W. Newhouse (unpublished data) in otoliths of steelhead from northern California waters.

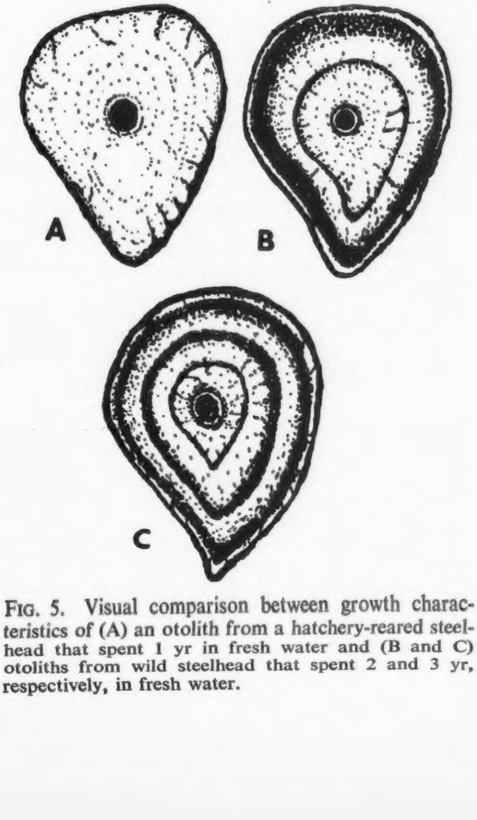


FIG. 5. Visual comparison between growth characteristics of (A) an otolith from a hatchery-reared steelhead that spent 1 yr in fresh water and (B and C) otoliths from wild steelhead that spent 2 and 3 yr, respectively, in fresh water.

In winter steelhead, which normally enter fresh water during the winter and spawn in late winter or early spring, the spawning check coincides with formation of the annulus. Though resorption is easily detected, the record of previous growth can be interpreted. But in summer steelhead, which enter streams during the summer or early fall and spawn in late winter or early spring, the stress of formation of sex products with little or no feeding appears to cause resorption over a prolonged period so that the growth record often is missing. In either case, growth of otoliths follows the ocean pattern after the fish return to the sea, and new growth follows the contour of the edge established by resorption.

The summer and winter races of steelhead were separable on the basis of diameters of nuclei of otoliths throughout the north–south range studied. Because the nucleus represents that portion of the otolith which develops during embryonic growth when nourishment comes entirely from the yolk, this reflects a quantitative and qualitative difference in the substances available to the young of each race. Bulkley (1967) found that steelhead from the southern part of their range tended toward higher fecundity than their northern counterparts because of additional stresses from the freshwater environment. The same trend might be expected as a difference between summer and winter steelhead because summer steelhead enter streams when flows are low and water temperatures are relatively high (Rousseau unpublished data) whereas winter steelhead enter streams when flows are higher and water temperatures are lower (Wagner et al. 1963). Egg counts substantiate this hypothesis. Bulkley (1967) reported an average fecundity of 3438 eggs per female (N = 73) for Alsea winter steelhead (mean FL = 64.9 cm); and Fessler and Wingfield, respectively, reported an average fecundity of 5529 eggs per female (N = 18) for Deschutes River, Oregon, summer steelhead (mean FL = 57.8 cm) and 4270 eggs per female (N = 28) for Siletz River summer steelhead (mean FL = 68.5 cm) (unpublished data). The smaller nucleus diameter of the otolith probably reflects the mechanism (increased fecundity) by which summer steelhead compensate for adverse conditions (prolonged residence in fresh water) to which winter steelhead are not exposed.

A similar quantitative and qualitative difference is reflected in the measurement of the first year of growth in fresh water. Hatchery fish typically exhibit no seasonal variation in otolith growth for their year of artificial rearing, whereas growth of otoliths from wild fish reflects seasonal cycles in food abundance.

Acknowledgments — This study was supported in part by a grant from the Sport Fishing Institute, Washington, D.C. Dr C. E. Bond, Professor of Fisheries, and Dr R. G. Peterson, Professor of Statistics, gave valuable suggestions for collection and analysis of data. Personnel of the Oregon Wildlife Commission, especially Dr H. H. Wagner and Mr R. Rousseau, cooperated in the investigation. Messrs J. E. Lannan, A. G. Ott, R. A. Scott, and D. L. Zeigler, former Research Assistants, helped in the collection and interpretation of materials. The assistance of Mr D. Miller, Fisheries Research Institute, personnel of the Washington Department of Game, and Mr C. Lyon, British Columbia Department of Fisheries, is greatly appreciated.

Andrews, R. E. 1959. Factors influencing the seaward migration of steelhead trout, *Salmo gairdnerii gairdnerii* Richardson, in the Alsea River, Oregon. M.Sc. Thesis. Oregon State College, Corvallis, Oreg. 90 p.

Bilton, H. T., and D. W. Jenkinson. 1968. Comparison of the otolith and scale methods for aging sockeye (*Oncorhynchus nerka*) and chum (*O. keta*) salmon. J. Fish. Res. Board Can. 25: 1067–1069.

Bruning, J. L., and B. L. Kintz. 1968. Computational handbook of statistics. Scott, Foresman and Co., Glenview, Ill. 269 p.

Bulkley, R. V. 1967. Fecundity of steelhead trout, *Salmo gairdneri*, from Alsea River, Oregon. J. Fish. Res. Board Can. 24: 917–926.

Clutter, R. I., and L. E. Whitesel. 1956. Collection and interpretation of sockeye salmon scales. Int. Pac. Salmon Fish. Comm. Bull. 9: 159 p.

Kim, W. S. 1963. On the use of otoliths of red salmon for age and racial studies. M.Sc. Thesis. Univ. Washington, Seattle, Wash. 63 p.

Kim, W. S., and T. S. Y. Koo. 1963. The use of otoliths for age determination in red salmon. Univ. Washington Fish. Res. Inst. Contrib. 147: 17–19.

Kim, W. S., and K. Roberson. 1968. On the use of otoliths of sockeye salmon for age determination, p. 149–168. *In* R. L. Burgner [ed.] Further studies of Alaska sockeye salmon. Univ. Washington Publ. Fish. (New Ser.) Vol. III.

Koo, T. S. Y. 1955. Biology of the red salmon, *Oncorhynchus nerka* (Walbaum) of Bristol Bay, Alaska as revealed by a study of their scales. Ph.D. Thesis. Univ. Washington, Seattle, Wash. 164 p.

Larkin, P. A. 1972. The stock concept and management of Pacific salmon, p. 11–15. *In* R. C. Simon and P. A. Larkin [ed.] The stock concept in Pacific salmon. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

Maher, F. P., and P. A. Larkin. 1954. Life history of the steelhead trout of the Chilliwak River, British Columbia. Trans. Am. Fish. Soc. 84: 27–38.

McKern, J. L. 1971. Steelhead trout otoliths for age, race, and stock analysis. M.Sc. Thesis. Oregon State Univ., Corvallis, Oreg. 55 p.

McKern, J. L., and H. F. Horton. 1970. A punch to facilitate the removal of salmonid otoliths. Calif. Fish Game 56: 65–68.

McMurrich, J. P. 1910. The life history of the Pacific salmon. Trans. Can. Inst. Toronto 9: 23–44.

1913. The life cycles of the Pacific coast salmon belonging to the genus *Oncorhynchus*, as revealed by their scale and otolith markings. R. Soc. Can., Ottawa. Proc. Trans. 1912, Ser. 3, Vol. 6, Geol. Biol. Sci. p. 9–28.

Ricker, W. E. 1972. Hereditary and environmental factors affecting certain salmonid populations, p. 19–160. *In* R. C. Simon and P. A. Larkin [ed.] *The stock concept in Pacific salmon*. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

Rollefsen, G. 1935. The spawning zone in cod otoliths and prognosis of stock. Rep. Norw. Fish. Mar. Invest. 4(11): 1–10.

Shapovalov, L., and A. C. Taft. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*) with special reference to Waddell Creek, California, and recommendations regarding their management. Calif. Dep. Fish Game Fish. Bull. 98: 375 p.

Wagner, H. H., R. L. Wallace, and H. J. Campbell. 1963. The seaward migration and return of hatchery-reared steelhead trout, *Salmo gairdneri* Richardson, in the Alsea River, Oregon. Trans. Am. Fish. Soc. 92: 202–210.

Withler, I. L. 1965. Variability in life history characteristics of steelhead trout (*Salmo gairdneri*) along the Pacific Coast of North America. J. Fish. Res. Board Can. 23: 365–393.

Comment on the Use of Ivlev's Electivity Index with Planktivorous Fish

W. JOHN O'BRIEN AND GARY L. VINYARD

Department of Systematics and Ecology
University of Kansas, Lawrence, Kans. 66045, USA

O'BRIEN, W. J., AND G. L. VINYARD. 1974. Comment on the use of Ivlev's electivity index with planktivorous fish. J. Fish. Res. Board Can. 31: 1427–1429.

Application to natural fish populations of Ivlev's equation of electivity, by which the degree of choice a predator exhibits in its feeding behavior is determined by comparing the proportion of a particular prey species present in the environment with the proportion present in the gut, has frequently depended upon the incorrect assumption that both predaceous fish and their zooplankton prey are evenly or randomly distributed throughout a water body. In fact, zooplankton tend to clump both vertically and horizontally, and the use of a vertically towed net to determine zooplankton density does not accurately indicate the zooplankton species composition available to a fish feeding at a particular location. Certain zooplankton sampling equipment also may show selectivity or may not capture particular species, so that a difference between the percent species composition of the plankton sample and that of the fish gut may be due to sampling bias rather than fish electivity. Consideration of these problems will enable field researchers to gather more accurate data concerning fish feeding electivity.

O'BRIEN, W. J., AND G. L. VINYARD. 1974. *Comment on the use of Ivlev's electivity* index with planktivorous fish. J. Fish. Res. Board Can. 31: 1427–1429.

D'après l'équation d'électivité d'Ivlev, on mesure le degré de choix dont fait preuve un prédateur dans son comportement alimentaire en comparant la proportion d'une espèce proie particulière présente dans l'habitat avec la proportion présente dans le tube digestif. Souvent, l'application de cette équation aux populations naturelles de poissons *est fondée sur l'hypothèse erronnée que les poissons prédateurs et leur proie zooplanctonique* sont tous deux répartis également ou au hasard dans toute la masse d'eau. En fait, le zooplancton tend à se grouper en masse compacte, tant horizontalement que verticalement, et l'usage d'un filet traîné verticalement en vue de déterminer la densité du zooplancton n'indique pas avec précision la composition spécifique du zooplancton accessible à un poisson qui se nourrit à un endroit particulier. De plus, certains échantillonneurs de zooplancton peuvent être sélectifs ou incapables de capturer une espèce en particulier, de sorte qu'une différence entre le pourcentage de composition en espèces de l'échantillon de plancton et celui du tube digestif peut être dû à un biais d'échantillonnage plutôt qu'à une électivité par le poisson. L'attention portée à ces problèmes permettra aux chercheurs sur le terrain de recueillir des données plus précises concernant l'électivité dans l'alimentation des poissons.

Received February 13, 1974
Accepted May 8, 1974

Reçu le 13 février, 1974
Accepté le 8 mai, 1974

IN the years since the 1961 publication in English of his classic *Experimental Ecology of the Feeding of Fishes*, Ivlev's work and particularly his equation of electivity has been a springboard for much comment and research in the area of fish ecology. With this equation, it is possible to determine the electivity, or degree of choice, a certain predator shows in its feeding behavior. Ivlev expressed this equation:

$$\mathrm{E} = \frac{r_i - p_i}{r_i + p_i}$$

where E = electivity, r_i = % composition of plankter *i* in the gut contents, and p_i = % composition of plankter *i* in the plankton sample. The result is an electivity index range from -1 to +1, with -1 indicating complete avoidance, 0 indicating no active selection, and +1 indicating complete selection.

Ivlev used the electivity equation to identify various components of prey selection by fish. He

Printed in Canada (J3318)
Imprimé au Canada (J3318)

conducted his experiments in large aquaria where the relative densities of various prey items were known absolutely (no subsampling involved) and where neither prey nor predator was likely to be distributed in clumps. Since then a number of researchers have applied Ivlev's approach in field studies of planktivorous fish feeding (Houde 1967; Siefert 1968; Werner 1969; Cramer and Marzolf 1970; Starostka and Applegate 1970; Hutchinson 1971).

By comparison with Ivlev's carefully controlled aquaria, however, a natural water body presents a number of serious problems in applying Ivlev's equation to predation on zooplankton. Neither zooplankton nor fish are likely to be evenly or randomly distributed throughout the horizontal and vertical expanse of a lake. Therefore, before the feeding electivity of a fish can be accurately determined in a natural situation, it is necessary to know how many zooplankton are present at a particular point and to know the length of time the fish has fed at that point.

Many species of zooplankton tend to clump at particular depths, and the depth of maximum density changes during the day and season (Hutchinson 1967; George and Fernando 1970; Cunningham 1972). Zooplankton may also clump horizontally as well as vertically (Hutchinson 1967; Patalas 1969; Starn 1971; George and Edwards 1973), and fish may migrate vertically to those depths at which they are most likely to find abundant food (McNaught and Hasler 1964; Narver 1970; Finnell and Reed 1969). It is therefore important in determining the electivity of fish to know the vertical and horizontal distribution of both the fish and the zooplankton species being studied.

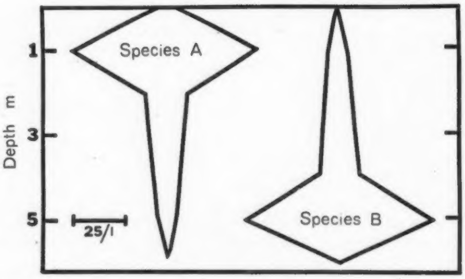


FIG. 1. Hypothetical vertical distribution of two zooplankton species. Both species have overall identical abundance with species A 9 times more abundant than species B at 1 m, with both species equally abundant at 3 m and species B 9 times more abundant than species A at 5 m.

TABLE 1. Electivities which would be calculated from a completely non-elective fish feeding at three depths (1, 3, 5 m). The hypothetical vertical zooplankton densities are as shown in Fig. 1 but the electivities are computed from the results which a vertical plankton tow would yield (equal relative densities).

Depth of feeding (*m*)	Apparent electivity	
	Species A	Species B
1	+.29	−.29
3	0	0
5	−.29	+.29

Figure 1 and Table 1 show a hypothetical situation in which the electivity of fish would appear to vary if zooplankton were considered to be randomly or evenly distributed. This is often the case when a vertical zooplankton tow is used because it tends to integrate the vertical zooplankton distribution. Figure 1 shows an assumed vertical distribution of two zooplankton species present in overall equal densities but with great differences in density at certain depths. A vertical tow taken from 6 m would show only that the relative densities of the two species are equal (25/liter) when integrated over 6 m, and fish electivity would be computed on that basis. However, only at 3 m are the two species distributed evenly. For this hypothetical situation, we assume that the fish to be studied shows no electivity when presented with these two prey species. However, Table 1 shows that several different electivities, depending on the depth at which the fish was feeding, would be calculated because of the clumped distribution of the zooplankton. That is, fish feeding at depths 1 or 5 m would appear to be selecting one prey species over another when actually the fish would be feeding on both species in direct proportion to their availability at each depth. A fish feeding at 3 m would show no electivity because at that depth the density of the two zooplankton species is identical.

In a lake the situation may be even more complex because zooplankton species densities change throughout the day and fish may feed at different depths at different times of the day (McNaught and Hasler 1964; Narver 1970; McDonald 1973). Also to be considered is patchy horizontal distribution; a single vertical plankton tow that either misses or includes an area of high density of a particular species does not give an accurate indication of zooplankton density (Wiebe 1971).

A second problem in determining fish electivity is the selectivity of zooplankton sampling equipment. It is well known from both freshwater and marine work that zooplankton vary in their tendency to be captured (Szlauer 1964) and that various species may avoid certain types of equipment (Barnes and Tranter 1965; Fleminger and Clutter 1965; Szlauer 1968; Aron and Collard 1969). In most fish electivity studies, however, investigators compare the percent species composition of the fish guts to the percent composition of the plankton sample and attribute any difference to electivity of the fish. Quite the opposite could be the case. An apparent positive electivity of the fish for a certain zooplankton species could be a negative selectivity of the sampling equipment for the same species.

These problems which may confound a fish prey preference study should be considered. Some workers have eliminated most of these problems (e.g. Houde 1967; McDonald 1973) but none has discussed these problems specifically.

Determining the depth at which fish are feeding may be the most difficult task. However, there are several possible approaches to this problem. Horak and Tanner (1964) and Hartman (1962) have used vertically strung gillnets and have assumed the fish were feeding at the depth of capture. Houde (1967) used a midwater trawl or 1 m diameter net towed at several depths and assumed the depth of feeding to be the depth of capture. Where possible the most accurate method would be to observe fish through the use of sonar for a fairly long time before capture (Narver 1970).

Where the depth of feeding is not known it would seem best if in studies of electivity researchers collect data on the vertical distribution of the zooplankton species involved. With such data the maximum and minimum electivities possible could then be computed and the importance of this potential problem to these investigations assessed.

Aron, W., and S. Collard. 1969. A study of the influence of net speed on catch. Limnol. Oceanogr. 14: 242–249.

Barnes, H., and D. J. Tranter. 1965. A statistical examination of the catches, numbers and biomass taken by three commonly used plankton nets. Aust. J. Mar. Freshwater Res. 16: 293–306.

Cramer, J. D., and G. R. Marzolf. 1970. Selective predation on zooplankton by gizzard shad. Trans. Am. Fish Soc. 99: 320–332.

Cunningham, L. 1972. Vertical migrations of *Daphnia* and copepods under the ice. Limnol. Oceanogr. 17: 301–303.

Finnell, L. M., and E. B. Reed. 1969. The diel vertical movements of Kokanee salmon, *Oncorhynchus nerka*, in Granby Reservoir, Colorado. Trans. Am. Fish Soc. 98: 245–252.

Fleminger, A., and R. I. Clutter. 1965. Avoidance of towed nets by zooplankton. Limnol. Oceanogr. 10: 96–104.

George, D. G., and R. W. Edwards. 1973. *Daphnia* distribution within Langmuir circulations. Limnol. Oceanogr. 18: 798–800.

George, M. G., and C. H. Fernando. 1970. Diurnal migration in three species of rotifers in Sunfish Lake, Ontario. Limnol. Oceanogr. 15: 218–223.

Hartman, G. F. 1962. Use of gill-net rollers in fishery investigations. Trans. Am. Fish Soc. 91: 224–225.

Horak, D. L., and H. A. Tanner. 1964. The use of vertical gill nets in studying fish depth distribution, Horsetooth Reservoir, Colorado. Trans. Am. Fish Soc. 93: 137–145.

Houde, E. D. 1967. Food of pelagic young of the walleye, *Stizostedion vitreum*, in Oneida Lake, New York. Trans. Am. Fish. Soc. 96: 17–24.

Hutchinson, B. P. 1971. The effect of fish predation on the zooplankton of ten Adirondack lakes, with particular reference to the alewife, *Alosa pseudoharengus*. Trans. Am. Fish. Soc. 100: 323–335.

Hutchinson, G. E. 1967. A treatise on limnology. Volume II. John Wiley and Sons, New York, N.Y. 1115 p.

Ivlev, V. S. 1961. Experimental ecology of the feeding of fishes. Transl. D. Scott. Yale Univ. Press, New Haven, Conn. 302 p.

McDonald, J. 1973. Diel vertical movements and feeding habits of underyearling sockeye salmon (*Oncorhynchus nerka*), at Babine Lake, B.C. Fish. Res. Board Can. Tech. Rep. 378: 55.

McNaught, D. C. and A. D. Hasler. 1964 Rate of movement of populations of *Daphnia* in relation to changes in light intensity. J. Fish. Res. Board Can. 21: 291–318.

Narver, D. W. 1970. Diel movements and feeding of underyearling sockeye salmon and the limnetic zooplankton in Babine Lake, British Columbia. J. Fish. Res. Board Can. 27: 281–316.

Patalas, K. 1969. Composition and horizontal distribution of crustacean plankton in Lake Ontario. J. Fish. Res. Board Can. 26: 2135–2164.

Siefert, R. E. 1968. Reproductive behavior, incubation and mortality of eggs, and postlarval food selection in the white crappie. Trans. Am. Fish Soc. 97: 252–259.

Starn, R. H. 1971. The horizontal–vertical distribution hypothesis: Langmuir circulations and *Daphnia* distributions. Limnol. Oceanogr. 16: 453–466.

Starostka, V. J., and R. L. Applegate. 1970. Food selectivity of bigmouth buffalo, *Ictiobus cyprinellus*, in Lake Pansett, South Dakota. Trans. Am. Fish Soc. 99: 571–576.

Szlauer, L. 1964. Reaction of *Daphnia pulex* de Geer to the approach of different objects. Pol. Arch. Hydrobiol. 12: 5–16.

1968. Investigations upon ability in plankton crustacea to escape the net. Pol. Arch. Hydrobiol. 15: 79–86.

Werner, R. G. 1969. Ecology of limnetic bluegill (*Lepomis macrochirus*) fry in Crane Lake, Indiana. Am. Midl. Nat. 81: 164–181.

Wiebe, P. H. 1971. A computer model study of zooplankton parchiness and its effects on sampling error. Limnol. Oceanogr. 16: 29–38.

First Record of the Margined Madtom (*Noturus insignis*) from Canada

P. J. Rubec and B. W. Coad

Department of Biology, University of Ottawa, Ottawa, Ont. K1N 6N5

Rubec, P. J., and B. W. Coad. 1974. First record of the margined madtom (*Noturus insignis*) from Canada. J. Fish. Res. Board Can. 31: 1430–1431.

The margined madtom (*Noturus insignis*) is recorded for the first time in Canada, from a stream tributary to Lac Lapêche, Québec (45°38.5′N, 76°12.2′W). Although its presence could be natural, it was probably introduced by the dumping of bait fish or the introduction of game fish.

Rubec, P. J., and B. W. Coad. 1974. First record of the margined madtom (*Notorus insignis*) from Canada. J. Fish. Res. Board Can. 31: 1430–1431.

Il s'agit de la première mention du chat-fou *Noturus insignis* au Canada, dans un cours d'eau tributaire du lac Lapêche, Québec (45°38.5′N, 76°12.2′W). Bien qu'il puisse s'y trouver naturellement, il a probablement été introduit en même temps que la boëtte rejetée à l'eau ou lors de l'introduction de poissons de sport.

Received February 8, 1974
Accepted April 19, 1974

Reçu le 8 février, 1974
Accepté le 19 avril, 1974

The natural range of the margined madtom (*Noturus insignis*) is considered restricted to Atlantic coastal streams of the United States and its presence elsewhere is interpreted as resulting from introductions (Taylor 1969). On August 8, 1971 four specimens of *N. insignis* were collected from a stream tributary to Lac Lapêche, Québec, (45° 38.5′ N, 76° 12.2′ W). On November 4, 1973 an additional 25 specimens were collected at the same location in a 5-m seine. The collections (NMC 73–142, 74–4) are deposited at the National Museum of Natural Sciences in Ottawa. From the collections, single specimens have been sent to the Laboratoire de Recherche, Service de la Faune du Québec, Montréal, to the Royal Ontario Muesum, Toronto, and to the Museum of Zoology, University of Michigan, Ann Arbor.

Scott and Crossman (1973) did not record this species from Canada. The present location is more than 201 km (125 air miles) north of its known distribution. Hubbs and Lagler (1964) noted that *N. insignis* (previously *Schilbeodes marginatus*) is present in stream tributaries in New York State entering Lake Ontario. Although listed in their keys for many years, it has not been found in other parts of the St. Lawrence drainage basin until this time.

Our specimens were collected in the riffle zone of a clear high gradient stream, under pebbles and small boulders, in a situation typical for the species (Taylor 1969).

Printed in Canada (J3310)
Imprimé au Canada (J3310)

Meristic and morphometric data were taken on 20 specimens following the methods of Hubbs and Lagler (1964) and Taylor (1969). The characters were within the ranges reported in Taylor's (1969) description of *N. insignis*. Other populations of *N. insignis* may occur in Canada and as it is most likely to be confused with the stonecat, *N. flavus*, the following key to species found in Canada is included.

1. Premaxillary band of teeth with lateral processes extending backwards........... *Noturus flavus*
   Premaxillary band of teeth without lateral backward extending processes.................. 2
2. Pectoral spines without serrae on posterior edges, mouth terminal with jaws of about equal length .... *Noturus gyrinus*
   Pectoral spines with serrae on posterior edges, lower jaw included........................ 3
3. Serrae of pectoral spines long and retrorse .... *Noturus miurus*
   Serrae of pectoral spines short and erect .... *Noturus insignis*

Although this occurrence of *N. insignis* could be natural, it is more likely due to an introduction. Taylor (1969) believes the populations of *N. insignis* outside Atlantic coastal streams most probably resulted either by dumping bait fish or while introducing game fish. It is a common practice for Pennsylvania anglers to take *N. insignis* north as live bait on fishing trips (R. M. Bailey, University of Michigan, personal communication). In western Québec it is illegal to transport live bait by land or air from the water being fished or to use species other than minnows as bait. If *N. insignis* was introduced it probably occurred some time ago when restrictions were not in force. Mr J. Harrison, a long time resident, has known of this population about 14 yr.

Acknowledgments — Dr D. E. McAllister provided the use of X-ray facilities at the National Museum of Natural Sciences, Ottawa and verified the identification. Dr R. M. Bailey, University of Michigan, supplied further background information. Mr G. Hamre assisted in making the collections. The National Capital Commission funded the survey.

HUBBS, C. L., AND K. F. LAGLER. 1964. Fishes of the Great Lakes region. Cranbrook Inst. Sci. Bull. 26: 213 p.

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

TAYLOR, W. R. 1969. A revision of the catfish genus *Noturus* Rafinesque, with an analysis of higher groups in the Ictaluridae. U.S. Nat. Mus. Bull. 282: 315 p.

PERSPECTIVES

Play it Again Sam — an Essay on Salmon Enhancement

P. A. LARKIN

WITH APPENDICES BY BLAKE A. CAMPBELL AND C. H. CLAY

Institute of Animal Resource Ecology
University of British Columbia, Vancouver, B.C. V6T 1W5

Contents

Abstract 1434
Résumé 1435
Preface (Why this was written) 1436
Introduction (We've been trying for 100 years) 1436
The General Justification 1438
    The Social Context (People like salmon)
    The Economic Context (Salmon are valuable)
    The Biological Context (Enhancement is possible)
What to Do (Mostly easy things — $200 million worth) 1443
How to Do It (A salmon development corporation) 1446
Conclusion (Start Now!) 1447
Appendix I. Economic factors to be considered before undertaking salmon-enhancement programs **B. A. Campbell** 1448
Appendix II. Salmon enhancement in countries other than Canada **C. H. Clay** 1451

Printed in Canada (J3372)
Imprimé au Canada (J3372)

Abstract

LARKIN, P. A. (With appendices by B. A. Campbell and C. H. Clay). 1974. Play it again Sam — an essay on salmon enhancement. J. Fish. Res. Board Can. 31: 1433–1459.

For more than 100 years efforts have been made to increase the abundance of the various species of Pacific salmon. The success of these ventures is largely a matter of conjecture because the scale of natural fluctuations is sufficient to mask the effect of human intervention. No well-defined program for salmon enhancement has yet been developed for British Columbia as a whole. This is mainly attributable to the pressures for providing protection for the existing stocks in the circumstances of an intensive fishery and increasing effects of other resource uses.

From a social point of view, salmon enhancement is a highly desirable activity. Salmon occupy a special place in the culture of residents of the Pacific coast. Salmon are economically valuable and salmon fishing provides a rich source of employment. Recreational fisheries are valuable but their true economic worth is difficult to judge. Benefit:cost ratios for salmon enhancement should be calculated for whole programs rather than for individual projects to obtain an approach consistent with the desirable strategy of development.

From a biological point of view, salmon enhancement is feasible. There is potential in the ocean for growing more salmon; many historic runs can be rebuilt and there are many opportunities for colonizing watersheds that are currently inaccessible to salmon. Harvesting of increased stocks of salmon could pose risks for natural stocks that are relatively unproductive. More effective management and development of new strains of salmon. may be required in these circumstances. Predator-control programs may in some instances be useful adjuncts to salmon-enhancement projects.

The types of enhancement activity that will most likely be rewarding are those that interfere least with the natural life history. Removal of obstructions, regulation of stream flow, construction of artificial spawning channels, are typical simple measures that involve less research information and less risk than rearing hatcheries.

Priorities for different salmon-enhancement projects involve consideration of the regional economic and social conditions as well as appreciation of the biological and physical problems. Regardless of priorities it is essential to a successful program that there be a commitment to continuity. Additionally, the total administrative complex for salmon enhancement should involve research components.

The execution of a successful salmon-enhancement program depends on the establishment of an agency with the single responsibility of salmon enhancement. The financing of a successful program should depend partly on revenues generated from the resource users, such as a tax on commercially caught salmon, a license for saltwater angling. Special grants from federal and provincial governments would also be appropriate. Regardless of the administrative arrangements, it is urgently necessary that provincial government involvement be more substantial than it is at present. With their control over resource management practices, the provincial government is in a vital position for maintenance of freshwater environments.

An investment of at least $100 million, and probably twice that amount, could be undertaken in a salmon-enhancement program that could be executed within 10 years. There are compelling reasons for starting on this enhancement program immediately.

Résumé

LARKIN, P. A. (With appendices by B. A. Campbell and C. H. Clay). 1974. Play it again Sam — an essay on salmon enhancement. J. Fish. Res. Board Can. 31: 1433–1459.

Depuis plus d'un siècle, on s'efforce d'accroître l'abondance des diverses espèces de saumons du Pacifique. On ne peut que conjecturer le succès de ces entreprises, car l'envergure des fluctuations naturelles est suffisante pour masquer les effets de l'intervention humaine. On n'a pas encore mis au point de programme bien défini de revalorisation des stocks de saumons pour l'ensemble de la Colombie-Britannique. Ceci est dû en grande partie aux contraintes qu'impose la nécessité de voir a la protection des stocks actuels dans le contexte d'une pêche intensive et des effets croissants d'autres usages que l'on fait de la ressource.

Du point de vue social, la revalorisation du saumon est fortement désirable. Le saumon occupe une place spéciale dans la culture des résidents de la côte du Pacifique. Il a une grande valeur économique, et sa pêche est une importante source d'emploi, surtout pour des gens qui, dans une certaine mesure, pourraient difficilement trouver du travail autrement. Les pêches récréatives ont de la valeur, mais leur impact économique réel est difficile à juger. On devrait calculer les quotients bénéfice/coût de la revalorisation du saumon pour des programmes globaux, plutôt que pour des projets individuels, afin d'en arriver à une méthode d'attaque compatbile avec une stratégie de développement désirable.

Du point de vue biologique, la revalorisation du saumon est chose possible. L'océan offre un potentiel pour un plus grand nombre de saumons; plusieurs montaisons historiques peuvent être rétablies, et il y a beaucoup d'opportunités de peuplement de bassins hydrographiques présentement inaccessibles au saumon. La récolte de stocks de saumons plus abondants n'est pas sans risques pour des stocks naturels relativement peu productifs. Il se peut que, dans ces circonstances, on doive faire appel au développement de lignées nouvelles. Des programmes de contrôle des prédateurs peuvent, dans certains cas, être des compléments utiles à des projets de revalorisation du saumon.

Les types d'activité qui ont le plus de chances de porter des fruits sont ceux qui causent le moins d'interférence au cycle biologique naturel. L'enlèvement des obstacles, le réglage du débit des cours d'eau, la construction de chenaux artificiels pour la fraie sont autant de mesures typiques simples qui impliquent moins de recherche et moins de risques que les établissements de pisciculture.

Le choix des priorités dans les divers projets de revalorisation du saumon doit tenir compte des conditions économiques et sociales de la région, aussi bien que des problèmes biologiques et physiques. Quelles que soient les priorités ,des engagements de continuité sont essentiels au succès du programme. De plus, le complexe administratif global chargé d'un programme de revalorisation du saumon doit comporter des éléments de recherche.

L'exécution d'un programme réussi dépend de la mise sur pied d'un organisme qui en a la seule responsabilité. Son financement doit provenir en partie des revenus engendrés par les usagers de la ressource, tels que taxes sur les saumons capturés commercialement et permis de pêche sportive en eau salée. Des subventions spéciales des gouvernements fédéral et provincial seraient également de mise. Quelles que soient les dispositions administratives, la participation du gouvernement provincial doit, de toute nécessité, être plus substantielle qu'elle ne l'est présentement. Contrôlant, comme il le fait, la gestion de la ressource, le gouvernement provincial occupe une position clef pour le maintien des habitats d'eau douce.

Un investissement d'au moins 100 millions de dollars, et probablement le double de ce montant, pourrait être consacré à un programme de revalorisation du saumon à être exécuté en l'espace de 10 ans. Il existe des impératifs sérieux pour que ce programme de revalorisation soit immédiatement mis en marche.

Received March 20, 1974
Accepted April 18, 1974

Reçu le 20 mars, 1974
Accepté le 18 avril, 1974

Preface

In midsummer of 1973, the Fisheries and Marine Service of the Department of the Environment decided to convene a workshop on enhancement of Canadian fisheries resources. As background material three papers were prepared: one by C. H. Clay of the Food and Agricultural Organization of the United Nations, entitled, "Resource Enhancement in Countries other than Canada"; another by D. B. Lister, entitled, "A Brief Review of Salmon-Enhancement Programs in Pacific and Atlantic Canada"; and a third on Pacific salmon enhancement, by myself, in the form of an essay called "Play it Again Sam."

This document is a revised version of the essay which retains the original appendix by Blake Campbell on Economic Factors. The revision has added, as an appendix, parts of the paper by C. H. Clay and, along the way, some notes have been added that reflect the discussions at the workshop held in Vancouver, January 29–30, 1974. Altogether, this document summarizes many facets of the complex business of enhancement of Pacific salmon.

The initiative of Mr K. C. Lucas, Senior Assistant Deputy Minister, and his staff in Ottawa, and the very considerable assistance and encouragement of the Fisheries and Marine Service in Vancouver, particularly of the Area Director, Mr W. R. Hourston, is cordially acknowledged.

Introduction

EXCERPTS from the Annual Reports, Department of Marine and Fisheries, for the years 1882–87.

" The question of a salmon hatchery on the waters of the Fraser continues to be agitated. By telegram from the Department, of 10th July last, I was asked to 'name the place on Fraser River best suitable for a salmon hatchery?' To this I telegraphed in reply, suggesting that, in a decision so momentous, the opinion of an expert from Canada, cognizant of all the requirements, should rather be called for. [1882]

" As instructed, I engaged, in October last, Mr Thomas Mowat, to choose a site on the Lower Fraser for the erection of a fish hatchery. The site has been duly selected at a point some four miles above New Westminster, on the opposite shore, where Messrs. B. Haigh & Sons have a cannery. ... With regard to this hatchery question I must again ask reference, should the question of success ever hereafter arise, to the substance of the remarks contained in several of my previous reports. [1883]

" A fish hatchery, measuring 100 × 40 feet, was built during the last season on the Fraser River. It will easily accommodate 3,000,000 *Quinnat* salmon eggs, or 5,500,000 *Saw-quai* salmon ova. By doubling the trays, double this number of eggs can be laid down. The catching of parent salmon began about the beginning of July, and by the close of the season 3,000,000 eggs were on the trays. The operations, although novel, were highly successful and reflect credit upon the officer in charge, Mr. Thomas Mowat. [1884]

" The premises upon which the assumed complaint was made to the Board of Trade, were founded upon a want of practical knowledge of the changes which take place in the color of the flesh and eggs of all salmon after leaving the salt water, and remaining for a time in the fresh water of the rivers. Whilst at their first entrance from the sea, the flesh will be found a deep red hue, it, nevertheless, begins at once to change in color, and deteriorates in quality, and when the time for spawning is reached, from September till November, or later in the season, the flesh becomes whitish and flabby, and the eggs become changed in appearance also.

This is such a well established fact amongst all persons practically acquainted with the nature and habits of the salmon the world over, that it will be unnecessary for further comment here. It is, nevertheless, to be regretted that, any 'assumed' complaints against the working of an Institution, which has been established in the interests of the fisheries of the Fraser River, should be made at the first commencement of its work, and particularly so, without any real or even intelligent cause being given for the allegation. [1885]

" It now behooves all persons engaged in this great industry, to do everything in their power to devise means to open other streams closed by mill dams or natural falls, for natural breeding, and also to increase the facilities for artificial propagation which, I am satisfied, will be of great value in assisting to keep up the supply of salmon in this river. [1886]

" The decrease in 1886, is attributed to the annually increased quantities of salmon packed on the Fraser River for the last ten years, but it is confidently looked for by many that the runs of fish will be more steady in the future from the assistance of re-stocking by artificial means. [1887]

Still another centennial can be celebrated in British Columbia, for it is now 100 years more or less since enthusiasms were first generated for enhancing the numbers of salmon. As was said then, and many times since, it should be easy to increase the numbers of salmon. In natural circumstances, there are large mortalities to the eggs and young from floods and droughts, and a variety of natural predators are attracted wherever the salmon congregate in the course of their natural migrations. The losses that occur in fresh water are not only apparent, but evidently man can intervene so that the losses may be avoided. It is also obvious that salmon do not occur in many of the river systems where they might be expected, nor are they equally abundant every year.

With these lines of argument, the salmon culture programs of the turn of the century were "spawned" with the promise of "millions more fish." The main effort went to hatcheries because salmon are easy to catch in streams, their eggs are readily fertilized, and their young are easy to raise. Along the whole of the west coast, hatcheries each year turned out many millions of salmon fry and fingerlings that were planted in their streams of origin. Also, fish were transplanted to other watersheds so that new populations could be established, or so that off-years could be filled in. Salmon were even planted in far away places like New Zealand and Maine. But hatcheries weren't the only aspect of the early programs aimed at increasing the numbers of salmon. Natural obstructions to migration were chopped and blasted, and where man-made obstructions occurred, they were removed (though not always in time to prevent serious damage). The most zealous fisheries officers and conservationists waged private wars on predators such as bears and squawfish. In many instances they extended their private efforts to rescuing stranded young salmon from riverside pools, brailling adults past obstructions, and most importantly, impressing on the general public the value of giving salmon a helping hand. In brief, there was a passion for activities that we now call salmon enhancement.

It is unfortunate that the sum effect of all of these activities was virtually unmeasurable. In the natural course of events, there were wide variations in the numbers of salmon from year to year, and from generation to generation, and much of this variation stemmed from fluctuations in weather conditions or from complex biological causes that even yet are not well understood. Moreover, the effects of the fisheries for salmon were only partially revealed by the gross statistics of catch. Even the most successful program might seem to have been fruitless because the salmon were caught anonymously at sea. In consequence, the effects of local and ephemeral efforts at salmon enhancement were lost in the much larger scale of numbers in the total system.

On some occasions the results achieved seemed to provide the necessary encouragement to further work, and there was always the underlying conviction that what was being done couldn't possibly be harmful. The momentum of salmon cultural work was thus carried into the thirties. At that time, there was a growing scientific activity that was critically examining all aspects of salmon management. In British Columbia, the most significant studies were those on sockeye salmon at Cultus Lake by Foerster and Ricker, and because their results were misunderstood and because of a zest for economies, the hatchery programs were discontinued.

Since World War II, the major efforts in salmon management have been directed to more sophisticated manipulation of regulations for the fisheries, with increasing attention to international aspects, and a major preoccupation with protection of salmon from the effects of development of other resources. From time to time there have been enhancement projects, in some measure spurred by the necessities for ameliorating effects of dams or water diversions, but there has been no large-scale systematic effort to artificially increase salmon production. The International Pacific Salmon Fisheries Commission has perhaps shown the greatest singleness of purpose with various projects designed chiefly for sockeye salmon, to facilitate migration, stabilize water flows, provide spawning channels, and to rehabilitate (or restart) runs. The Fisheries and Marine Service (formerly the Fisheries Service, formerly the Department of Fisheries) and the former Fisheries Research Board, have had a much more diffuse program involving construction of pink salmon spawning facilities on some coastal streams (e.g. Jones Creek, Robertson Creek), a project for stabilization of flow of the Qualicum River, a project for greater production of salmon fry at strategic points on Babine Lake (Fulton River and Pinkut Creek), and recently, construction of a hatchery for coho on the Capilano River, with another scheduled for Quinsam on Vancouver Island. There were, of course, many smaller projects for bypassing obstructions or exploring particular fish cultural opportunities. Since 1945 there have been 36 projects on the west coast and their capital cost was $25 million. The Fish and Wildlife Branch of the British Columbia Provincial Department of Recreation and Conservation has, from time to time, collaborated with the federal agencies and has engaged in small steelhead rearing programs; but, in general, their enhancement programs for anadromous fish have been trivial.

It isn't easy, of course, to see what people have in mind or have had in mind, but, judging by what has been published, it would seem that a well-defined program for salmon enhancement has not yet been developed for British Columbia as a whole. In part, this is understandable, because the pressures for protection and management have been heavy and the techniques of enhancement were on a shaky scientific basis. Indeed, some of the attempts at enhancement have been so embarrassingly unsuccessful that it is fortunate they were envisaged as "trials" or "experiments" from the outset. It is not untrue nor unkind to observe that many of the projects have been launched with the best preparations possible, but with many prayers.

At the present juncture, the pressures for regulation remain and the pressures for protection are accelerating. The streams of British Columbia are rapidly changing in character as a consequence of other resource development. At the same time, the value of salmon in world markets is increasing at a phenomenal rate. The necessity for considering enhancement as a major activity is demonstrable.

This document discusses some of the strategic considerations for salmon enhancement in British Columbia. Many persons and agencies contributed ideas and suggestions. Deliberately, it has been written in the form of an essay, rather than as a scientific analysis of the effectiveness of enhancement procedures. There is a large and conflicting technical literature, and most of it has already been shuffled sufficiently. There is a much larger body of information in unpublished government reports and much of this has aged sufficiently to be beyond useful analysis. In consequence, this document is essentially a subjective appraisal of the present situation and prospects. It would be expected that any technical programs carried out would be preceded by appropriate technical background assessments.

The General Justification

To the simple-minded (fishermen and biologists), it is desirable to have more salmon to catch. The essence of their thinking is that if two is good, three is better. The more sophisticated (businessmen and economists) are quick to point out that, where demand is limited, there may be no merit in greater supply — three are better only when worth more than two. And where demand is indeed unlimited, there is merit to increasing supply only where the revenues exceed the costs. Three are better only when the third costs less than it is worth. With the government paying the costs, the businessman or fisherman may have enthusiasms where the benefit: cost ratio is marginal, whereas the economist, with wider-ranging perspectives, is usually inclined to consider what he calls the whole social investment strategy. But, regardless of benefit: cost ratios, whether favorable or not, an even more sophisticated analysis (by politicians and sociologists) may suggest reasons for action that defy the optima of biological or economic technology. It is accordingly necessary to justify expenditures on salmon enhancement in what may seem a reverse order, first evaluating the social context, then the economic costs and benefits, and finally considering the biological nuts and bolts.

THE SOCIAL CONTEXT

Perhaps the greatest sociological merit of salmon enhancement is associated with the romantic notions that people have about an edible fish that goes to such conspicuous trouble to spawn. Many residents of British Columbia would be pleased to see their taxes spent on salmon because they feel that salmon are part of a west coast inheritance that should be preserved at all costs. It is part of the mystique of the Pacific that salmon come home to spawn, and it is a part of every child's education to join in the aboriginal traditions of reverence for salmon. For cultural reasons alone, salmon enhancement is much to be desired.

More prosaically, salmon fishing provides a source of employment. It is a labor intensive activity. It is an especially useful kind of employment for people who have a passion for salmon fishing, or for people who find it difficult to make a living in other ways. Many of these people would be a potential social problem and would perhaps become a source of social costs if there were no salmon fishing. This isn't true, of course, of every fisherman, because many are flexible entrepreneurs who could turn their hands to other kinds of fishing or other kinds of employment. But the fact remains that many of the salmon fishermen are emotionally or socially, irrevocably tied to salmon fishing as a main source of livelihood.

In addition to the arguments based on the general popular sentiment, employment opportunities, and the social immobility of the professional salmon fishermen, there is the persuasion of the sport fishermen. With a lesser element of individual economic necessity, this diverse group of amateurs exploit salmon as a source of recreation, supplementary income, and food. They are characterized by a greater measure of enthusiasm for salmon than is typical of the general populace, a lesser measure of dependence than commercial fishermen on salmon as a source of bread and butter, but, above all, an almost religious conviction that salmon enhancement is one of the most exalted activities of mankind.

In recent years, emphasis has also been placed on the social values of investing in renewable resources of food in a world that shows increasing prospect of protein shortages. This argument is not particularly impressive in substance because salmon are collectively a small contributor to total world fish catches, and as far as protein goes, we could get 10 times more by harvesting what salmon eat (provided people would eat it). But the substance of the argument is perhaps not so important as its intent. However trivial the contribution of salmon may be, it is a contribution, and it does not behoove us to subtract it from the total because we have alternatives. On the contrary, every bit counts, and British Columbians generally, derive satisfaction in reflecting on the food that their ocean produces.

The foregoing are everyday facts of life on the west coast, and they create a favorable social climate for salmon enhancement. They also provide a fertile base for informed criticism of whatever is undertaken. There is little danger that a vigorous program will lack the element of public interest that is the vital prerequisite of public participation. It is hard to imagine otherwise.

On a more technical plane, it is less easy to justify salmon-enhancement programs. The vague platitudes of the man on the street may be sufficient to motivate short-term political action, but the implementation of major long-term programs requires a better documentation of social benefits. Unfortunately, the type of studies necessary have never been done, and human nature being what it is, it may never be possible to do them adequately. Extensive sociological analysis could no doubt lead to a more informed assessment of the social impact of salmon-enhancement projects, but the underlying assumption would probably be that human behavior would remain constant. It is a matter of reasonably sure conjecture, but nevertheless conjecture, that public enthusiasm for salmon will persist and perhaps grow, that greater incomes for salmon fishermen will be associated with social benefits and that sport fishing for salmon will continue to be a common recreation. In addition, attitudes concerning salmon are linked to other concerns. Each investment in salmon protection or enhancement may reinforce the sanctity of river systems, making them less usable as sewers for wastes, generators of hydro power, or sources of water for domestic and agricultural uses. Additionally, the greater and more widespread the salmon resource, the more the restrictions on forestry practice. The opportunity costs may thus seem to exceed the salmon benefits. Where there are potential social pressures for employment in secondary industry or for accommodating larger populations however employed, an investment in developing salmon production may be argued to be unwise in the long term.

These kinds of arguments are especially to the fore in the circumstances of international agreements for harvesting salmon. At present, the United States shares sufficiently in the catch of Fraser sockeye and pink salmon that investment in salmon enhancement must pay off when Canada shoulders all the opportunity costs, half the enhancement costs, and gets only half the benefits.

Most of these arguments are essentially the typical parade of reasons for doing nothing. Any major change in public attitudes toward salmon does not seem to be in the books until at least that day when the conservation-minded younger generation is largely in the grave. There are more salmon enthusiasts now than ever before. The value of salmon as a resource is demonstrably greater than the forest crop with which it may compete because it takes 70 or more years to grow a crop of trees, but only from 2 to 5 to grow a crop of salmon. Moreover, we may well need salmon at some time in the future when we won't use trees. Industrial growth of British Columbia will generate greater recreational demand for salmon fishing. The trend of international negotiations is for Canada to get the equivalent of total Fraser production.

Thus, in the total social context, although it is difficult to convincingly rationalize any course of action from the present point of vantage, it would seem a sound decision to embark on a vigorous salmon-enhancement program. Within a relatively short time span, salmon-enhancement work will adequately reward investments. It is much wiser to seek the payoff now than to get bogged down in considering the ultimate social destiny of British Columbia. The immediate reality is that the increased development of British Columbia seems inevitable, and the chores of maintaining salmon runs at present levels look to be formidable. In the absence of positive steps to increase runs, the prospect is for an attrition of present stocks, with all of the potential social dislocations entrained. If only to forestall troubles and to preserve future options, salmon enhancement is called for now. More positively, the challenge of increasing salmon populations is in tune with the tenor of the times. British Columbians like salmon, want to keep those they have, and wish to have more. A vigorous enhancement program responds to these desires, offers better incomes and more employment, with emphasis on people of low social mobility. Politically, salmon enhancement is a "natural."

THE ECONOMIC CONTEXT

On the more narrow ground of economics, it is fairly straightforward to enumerate some of the factors to be considered in salmon enhancement work (see Appendix I by B. A. Campbell). Salmon is a luxury food for which there are strong markets in hard currency countries. Demand has been consistently good and in recent years has been reinforced because affluence is more widespread. Supply, by contrast, has remained fixed, or has declined. In the North Pacific as a whole, present-day catches are about half those of the 1930s. The general pattern of decline has not been as marked in British Columbia. In consequence, we are in a strong marketing position and salmon is an important source of export income. There is every prospect that this situation will continue. The decline of Atlantic salmon fisheries and the increasing evidence of poor management of Asian and Alaskan stocks can only mean a virtually unlimited market for British Columbia salmon.

A major concern of economists who have studied the salmon fisheries is centered around the question of public ownership. With no restriction on entry into the fishery, competition has led to overcapitalization. With the rigorous regulation of the catch, it has been inevitable that the fishing time has been reduced. Over much of the coast, fishing is permitted only for 2 or, at the most, 3 days per week. Rather evidently, the fishing power of the fleet is at least twice that necessary for taking the permissible catch. Added to this overcapitalization is the substantial continuing expenditure that is required for sophisticated management and adequate protection. In sum, it has appeared to economists that the salmon fisheries generate no new economic wealth, and in some instances may even be a deficit enterprise.

Aside from the social naiveté of this line of argument (how many of our kinds of human activity generate revenue?), the fact is that the value of salmon is increasing so rapidly (almost phenomenally) that the industry is at present a strong positive earner. Programs of license limitation, which are already underway, could no doubt improve the existing situation. But there is obviously some optimum number of fishing units and the optimum may be substantially larger than has previously been appreciated.

If present trends continue, the average earnings of salmon fishermen and of the salmon industry as a whole will accelerate so rapidly in the next decade that there will be strong pressures to halt the trend of license limitation. It is already happening. In 1972, earnings of $25,000 for the salmon season alone were not uncommon. Most to the point is to stress the positive side — there already exists a fishing and processing capacity that is sufficient to handle the results of any foreseeable enhancement program. There will be no necessity for large new investments in gear and facilities for some time to come.

The economics of the sport fishery are still essentially a matter of quasi-religious assessment of the dollar value of what people do in their spare time. The best line of approach is probably to try to find out empirically how to maximize the product of the number of fishermen and what they will pay for a license. It is then relatively unimportant (to an economist) to know how many fishermen are actually involved or who they are. There are some obvious political problems in such a trial-and-error attack on the sport-fishing revenue problem, and in consequence, the usual approach is to collect large amounts of information in the hope that statistical manipulation based on arbitrary assumptions will give some notion of economic value. Most of these kinds of estimates are, of course, controversial. Nevertheless, they do tend to place relatively high values on sport-caught fish, usually several times higher than for commercially caught fish. In consequence, sport fishing is increasingly promoted, sometimes even at the expense of commercial fishing. Bearing in mind that it requires a commercial sacrifice of about 10 fish to enable a sport fisherman to catch one fish (anglers are very inefficient), it would seem logical to expect a more rigorous demonstration of sport fish values before the preferential treatment of anglers is overdone. Moreover, there would seem to be good justification for a saltwater angling license as hard evidence of the demand by society for use of a resource. It is unfortunate that more research has not been done on these questions, for it may well be that the romance of salmon means much more or much less than we imagine in economic analyses. But, to state the status quo, there can be little doubt that anglers today are convinced by the economics of the economists they consult, and as voters would favor lavish expenditures.

Economics can be invoked at several levels in evaluation of salmon-enhancement programs. Benefit:cost ratios should, of course, be considered for individual projects (see Appendix II by C. H. Clay), but should not be the overriding factor in establishment of priorities except where all other factors are relatively equal. There are advantages, on purely technical grounds, in trying many techniques, so that an added benefit labelled "valuable experience" might tip the scales in favor of an otherwise marginal project. Many other examples come to mind for which intangible benefits may weigh heavily in setting priorities. The most important benefit:cost analysis is probably that which can be constructed on an aggregate scale, in which the costs of a group of projects over a series of years are related to the aggregate benefits. In this way the costs of the fishery and the costs of regulation and research can be adequately evaluated rather than in the arbitrary way that is necessary for single-project analysis. Additionally, an aggregate approach is more consistent with an appropriate strategy of development, which may well require some costly projects with low immediate benefits as integral parts of a long-range scheme of great benefit.

THE BIOLOGICAL CONTEXT

It might seem quaint to a non-biologist to question whether producing more salmon is good for the salmon, but natural systems being what they are, there is always a good chance that our best efforts will turn out to do more harm than good. For example, it is now widely recognized that the enthusiastic transfers of the hatcheries of yesteryear may have widely distributed many fish diseases. It has also been suggested that transfers may dilute the genetic characteristics of native strains, making them less well adapted and thus more vulnerable to many kinds of natural or human-induced disaster. There are evidently some restraints on salmon-enhancement programs.

From a purely quantitative point of view, the obvious question is whether the ocean can support more salmon. It has been suggested from time to time that, because different groups of stocks use different parts of the Pacific for rearing, there may be a limit to the total number of salmon that can be produced from any group of streams. Increased numbers would only result in greater mortality and/or a smaller average size of adult fish. Additionally, because there is some overlap in marine rearing areas, there is the possibility that very large runs of fish, e.g. in the Fraser, would cause a reduction in size of runs to the Skeena. The available evidence does not suggest there is any reason for substantial concern in this regard. From time to time there has been evidence that the presence of a large number of pink salmon may adversely affect sockeye growth and survival, and that large runs of either species may be associated with somewhat smaller average fish. But total production is larger when the total number of fish is larger; in addition, there are many instances in which these effects have not been observed. In general, it seems likely these effects would not occur on a scale sufficient to cause concern unless the total abundance of salmon were 2 or 3 times greater than it is now. In brief, there seems to be lots of headroom for expansion of salmon populations before the rearing areas of the ocean become a limiting factor.

This conclusion is scarcely surprising when one reflects on historic catches from British Columbia-based stocks, and on the conspicuous absence of some of these stocks in present-day catches. The Quesnel run, for example, is still far from its recorded size shortly after the turn of the century. Chum salmon runs are well below historic levels over much of the coast. An obvious plank in a salmon-enhancement platform is the restoration of these formerly large stocks.

Equally obviously, there are many evidences of natural accidents that have wiped out runs of salmon. For example, there are no anadromous stocks in the Great Circle chain of lakes (Tweedsmuir Park), or Kootenay Lake, though these systems once supported sea-run fish. Being now behind dams, these systems may be a poor prospect, but there are literally dozens of other examples of streams or lakes that represent natural catastrophes that can be undone. Additionally, there are an equal or larger number of situations where systems that have never supported salmon can be opened for colonization. British Columbia provides lots of opportunities for improving on nature.

A more serious concern is how to harvest increased runs of salmon without endangering natural stocks. Prevailing theories of salmon population dynamics indicate that the best strategy of harvesting is always to remove the surplus above an optimal escapement. By artificial means, the production from any escapement may be increased and the optimum may be changed so that greater catches can be taken. The key question is likely to be, "Can the greater harvest be taken without, at the same time, overharvesting stocks that are taken in the same fisheries"? It can be imagined that an extraordinarily efficient spawning channel or hatchery could support a fishery that took over 90% of its production of adults. But there are few, if any, natural stocks that can support this rate of harvest. It would thus be necessary to catch the hatchery fish at times and in places where there were no natural stocks intermingled.

The problem of harvesting the new production from enhancement programs is essentially an extension of existing problems of joint harvesting of natural stocks. It has been increasingly apparent in recent years that the intense fishery is slowly but irrevocably eliminating less productive natural runs. It seems difficult to imagine how this trend can be reversed. With so many individual stocks, it is technically impossible to manage each separately unless the harvest is on the spawning grounds, by which time the value of salmon as food is greatly reduced, if not negligible. It thus seems an inevitable long-term consequence of a heavy fishery that the least productive stocks will have to be sacrificed if the most productive are to be utilized, and that salmon-enhancement programs, if they are successful, will accelerate the process.

The obvious challenge to the manager is to develop new, more productive strains to take the place of depleted populations of natural streams, strains that may be said to be better adapted to the circumstances of harvesting. By the same token, it may be increasingly necessary to develop strains that can cope with the other circumstances of human activity, which include changes in regime of many of the physical and chemical characteristics of streams. Salmon enhancement with an emphasis on genetics thus emerges as a necessary activity to maintain present harvests, as well as a potential source of greater harvests.

On the grand level, there can be little doubt about the biological viability of salmon enhancement, but on a more detailed level there are other potential bogeymen. For example, in considering any tampering with a natural biological system, it is customary to speculate that the greater production will be compensated only by natural mortality of one kind or another — predation by squawfish, trout or dogfish, greater incidence of disease, and so on. These kinds of concerns are, in general, more serious when the organism concerned is relatively rare. For instance, an attempt to introduce Pacific salmon to the Atlantic may well fail because of some complex biological interaction.

Where the species concerned is ecologically dominant, as is the case with Pacific salmon in the Pacific, there is greater reason to believe that greater production at an early stage in the life history will result in greater production at the adult stage, at least in those systems that are currently relatively underpopulated. Nevertheless, there is considerable evidence to suggest that when populations of salmon are at very low levels, predation can be an important factor in preventing their return to high levels of abundance. Without going into the details of why this should be so, it has led to the frequent suggestion that any attempts at rehabilitation should be coupled with predator removals, or attempts at rehabilitation should be on a scale large enough to satisfy the predators as well as to produce a surplus sufficient to repeat the process in future generations.

These considerations lead logically to the possibility that predator-control programs may provide a simple way of increasing the production of large, existing runs. Where a seal or a killer whale catches a salmon in the immediate vicinity of a fisherman's net, there certainly seems to be some logic in immediate local control of predators. But, on a larger scale, predator-control programs may be dubious exercises. There is a multiplicity of natural predators and their interrelations with their various prey organisms are not well understood. In this field of speculation, one man's mathematical model is perhaps as good as the other's, but it does seem safe to surmise that an increase in salmon production would not all be cropped by an enlarged population of predators that were feeding more heavily. Perhaps the best answer to these speculations is that we will find out the answer only by experiment, and that any program of salmon enhancement should be coupled with a searching program of experimentation and evaluation.

The same may be said of most of the foregoing. Although it is straight forward to make convincing arguments about the biological potentials in salmon enhancement, it is abundantly evident from a century of failure that it isn't easy, or if it is easy, the results aren't abundantly evident. It is therefore important in considering investment in salmon enhancement, to clearly appreciate the character of the risks. The element of uncertainty in present predictions of salmon abundance comes largely from the year to year variation in climatic conditions. Because the effects on salmon are multiplicative, there is a very wide spread of returns for any given escapement. The implication for enhancement programs is that results may be discouraging for a series of years, but then an explosive success may more than make up for the years of poor return. The process is akin to a gambling game in which there is an equal chance of doubling your stake or having it cut in half. With enhancement, the odds are shifted so that there is a smaller chance of losing and a bigger chance of winning. You may have to wait a while for the "lucky streak" that gives you the average annual payoff that you are inevitably going to get.

This gambling aspect of enhancement programs has been treated inadequately in most justifications of projects, and it has implications for the strategy of investment. In present circumstances, with slow attrition of existing stocks there is a decreasing stake to gamble with. It is thus doubtful whether we should wait for positive demonstration of benefits from a few projects before investing on a larger scale. Additionally, to be certain of an immediate payoff, it is necessary to make many investments so that a few phenomenal successes will more than compensate the many small failures.

The present pattern of salmon enhancement on the British Columbia coast brings to mind a picture of a gambler who is in a house where it is policy to let the customer win, but where the gambler is playing with the two-bit slot machine while his take is gradually being dissipated in the others entertainments that the house provides. To get the benefit of the house rules, it is desirable to invest a lot in the many games with the big payoffs.

The gambling metaphor is also useful as a general guide to the best kinds of techniques to use in salmon enhancement. Where the technique is simple and obvious, it is most likely to pay the safest dividend for the investment because it involves the smallest chance of having inadvertent side effects. For example, the removal of a blockage in a natural stream leaves virtually everything to the fish — it simply opens up more environment for them to exploit. This is the way salmon colonized streams originally, and there are many well-documented instances in which it has been observed to work in recent times. The reoccupation of upstream pink salmon spawning grounds following the Hell's Gate fishway construction is a conspicuous example. Artificial spawning grounds are only a small step more complex, though as anyone expert can testify, there are many technical problems in their construction and maintenance. The most risky, most expensive, but potentially most rewarding projects are those that involve a complicated and extended human intervention in the natural salmon life history. Thus, hatcheries that produce yearling and older smolts of coho and sockeye are almost as notorious for their phenomenal failures as for their (apparently?) phenomenal successes. To some extent they may compensate for their erratic performance by their strategic contribution to a local fishery or by their appeal as "tourist-come-education" institutions; but in general they are akin to the roulette wheel in a gambling house and there are many less glamorous but more certain ways of investing.

The realization that the less you interfere the more likely you are to help, has been slowly gaining in strength over the past 50 years of salmon cultural activity, and perhaps reflects that early attempts were too ambitious in relation to the contemporary knowledge. It might be observed, somewhat ruefully, that if 50 years ago we had concentrated on removing obstructions and constructing works to regulate stream flow, and then building artificial spawning channels, we might now be ready, with strong salmon runs, to take the next steps into hatchery work. As it is, we have a fund of mixed experience about hatcheries that we acquired the "hard way," and we have a backlog of "simple" chores just to get our runs back to what they used to be. And the simple chores aren't so simple now, because the streams are used for many other purposes.

Tears aside, it is useful to indicate that much of a salmon enhancement program need not be flashy nor sophisticated. The simple provision of a natural environment for salmon is sufficient to pay dividends. That's what biology is all about.

Long distance transplants are a special class of salmon enhancement activity that is perhaps best likened to lotteries. Every now and then, over the past 100 years, Pacific salmon have been introduced to far distant places — to the North Atlantic (from both the North American and Asian sides), to Chile and New Zealand, and into Hudson Bay and the Great Lakes; and less exotically, up and down the Pacific coast from Alaska to California wherever there seemed a wide open possibility (such as even-year pink salmon south of Johnstone Straits). Judged by the results, the chances of success in these ventures are quite small, and as is the case for many lotteries, the outcome is often unexpected, and even hilarious. For example, a hatchery at what is now Thunder Bay, was used as a way station for pink salmon that were introduced into streams tributary to Hudson Bay. Two years later there were no returns to Hudson Bay, but some adult pink salmon turned up in streams near the hatchery! This led W. E. Ricker to observe that, to get fish into Hudson Bay, perhaps we should set up a way station hatchery on Hudson Bay as a base for introducing pink salmon into the Great Lakes! Quite frequently, too, "wide transplant" experiments have shown an initial success, but have subsequently fizzled out (the Russian transplant to the Barents Sea,[1] the US transplant to Maine, the Canadian transplant to Newfoundland).

There are many theories about transplants, but to date they are apparently not much more successful than the magic formulae that people use who buy lottery tickets. But the moral should not be to abandon transplants as crazy gambles. In general, the stakes are low, and in contrast to lotteries there is a chance that with each attempt we may learn something that will enhance the chances of success the next time around.

What to do

If there were only one species of Pacific salmon, only one river system in which they lived, only one kind of fisherman, and no other uses for streams, it would be fairly easy to decide what was the first priority for a salmon enhancement program. As it is, a multitude of biological, economic, and social factors combine to make the decisions about priorities particularly complex. A good way of approaching the problem would be to invest in a continuing "policy workshop" that could explore the potential interactions, identify the weak points in perception, and using the power of contemporary decision-making theory, probably resolve the issues somewhat more clearly. This kind of thinking is already underway and there is every expectation that the problems of setting priorities by quasi-quantitative methods will be partially resolved in the near future.

---

[1] The 1973 season showed a good return in the Barents Sea. Perhaps a selection has occurred that will lead to a permanent population (W. E. Ricker, Department of the Environment, Fisheries and Marine Service, Nanaimo, B.C. personal communication).

In the absence of a systematic treatment of the problem, there are at least some points that can be identified as important in establishing priorities, and they are probably sufficient for "getting on with the job" at once. First, the whole of the B.C. coast should probably not be considered as a unit as far as fishermen are concerned. Although it is true that the tendency in recent years has been to increasing mobility, both in the fishing activity *per se* and in the choice of a place to live, much of the fleet remains parochial. The major feature is the relatively fixed location of native fishermen in remote areas. From one social perspective, then, enhancement might do the most good in the central, northern, and Queen Charlotte Islands areas. Additionally, these areas are relatively undeveloped both socially and economically, and salmon enhancement programs would contribute to local prosperity. A second consideration is that the whole of the B.C. coast is not the same in the context of the "interception" problem. Investments on salmon enhancement on the Fraser, and to some extent the Skeena, are potentially subject to a greater "cut off the top" from the U.S. fleet, until some form of quid pro quo agreement is achieved. Again, the central and northern areas would seem preferable.

Directly contrasted to these lines of argument is the desirability of increasing sport fish availability in densely populated areas, which implies major investment in the Vancouver–Victoria region, and, looking to the future, perhaps the Prince Rupert area. In this regard it is only fair to note that a significant segment of the present catch of coho and chinook salmon in the Strait of Georgia and the Strait of Juan de Fuca is the product of hatcheries in the State of Washington. Contributions to the "kitty" from the Canadian side seem appropriate in the interests of good international relations, especially since it would strengthen our case at the bargaining table.

Another whole set of considerations relates to the relative economic value of the five species of salmon. Pink salmon are relatively low in value, but they mature at age 2 and give the quickest return on investment. They pose problems of adequate harvesting when they are abundant and inclined to be small. They tend to stray more than the other species from their stream of origin. Ideally, pink salmon enhancement should probably be done on a broad regional basis rather than intensively in small local areas. Chum salmon are also relatively low-valued, though in recent years the market for chum salmon eggs has been strong and increases the value of the average (i.e. both sexes) chum by 17 ¢/lb. Sockeye are more valuable but in British Columbia require 4 or 5 years to mature. Coho and chinook have substantial but variable value, depending on where and when they are caught. These two species are the backbone of the sport fishery, and their value may be correspondingly high.

A third set of factors is related to the differences in the life histories of the five species. Pinks and chums go to sea as fry, so there is no need to provide substitute or enhanced environments by controlling summer water flows, or to provide rearing facilities. For pinks and chums, simple enhancement techniques are all that are reasonably available to us, and except for the possible addition of predator control, all that are necessary in the freshwater part of the life history. Coho and sockeye both require a year of freshwater residence so enhancement must take cognizance of where the bottleneck may be in production. For example, coho are usually limited by the summer flows in streams and it is usually senseless to increase fry production and then to let nature take its course. For coho, the best enhancement practices are: 1) make new stream areas accessible (or plant fry in inaccessible places); 2) regulate water flows; and 3) rear fish in hatcheries for the whole first year. Similarly, production of sockeye fry by spawning channels or hatcheries is only sensible where lake rearing areas are understocked and, where they aren't, to concentrate on such techniques as: 1) fertilization to increase natural productivity; 2) predator control; 3) making new lake systems available; and 4) hatchery production for the whole first year. Chinook salmon may require some hatchery holding and feeding before their seaward migration, but because they generally are relatively rare and widespread in larger streams in B.C., enhancement for them alone is likely to be concentrated in a few local areas. Chinooks will generally benefit most from good conservation practices, and their production may be increased as a side effect of enhancement programs for other species.

Associated with these life history characteristics are the costs and risks associated with prolonged rearing and complex handling procedures. The simple provision of fishways past obstacles, augmented flows, or of artificial spawning channels, is much more likely to result in returns, whereas the rearing hatchery is more likely to be costly and to suffer mechanical breakdown or biological complications (disease especially) though it promises big returns. The need for research strengths in nutrition, disease, behavior, and physiology is very much greater where hatchery-like practices are attempted.

The hierarchy of priorities is thus not easily constructed, and for a variety of different combinations of reasons, several alternatives appear equally attractive. The best pattern of investment would thus seem to be one that was addressed to a wide range of possibilities. This is especially the case when the whole problem is viewed as a single research and development enterprise. There is no single technique that can be pointed to as a dead certainty to succeed at a specified rate. Some are obviously in the class of lotteries, and though the returns could be prodigious, at this stage it would not seem wise to invest a large amount in such ventures as transplants to Newfoundland, or transplants to establish off-year runs of pinks. These kinds of enhancement exercises are appropriate subjects for imaginative research and modest experimental trials. Coho and sockeye hatcheries are a bit more firmly based in an experience which is sufficient to encourage the former and discourage the latter, at least until more research can provide better understandings (particularly needed is research on sockeye population genetics, diseases, and behavior). Chinook hatcheries are probably a good investment in choice situations, if the experience on the Columbia River and the United States' hostility to Canadian troll fleet activities are reliable guides. Pink and chum enhancement by construction of stream flow control works and/or artificial spawning grounds, looks like a solid investment. Removal of obstructions to migration looks to be a gilt-edged prospect wherever it can be undertaken.

Regardless of the detail of the projects that are undertaken, it should be clearly recognized that there is not much point in undertaking a salmon-enhancement program unless there is a commitment to continuity. Looking back on the history of salmon enhancement in British Columbia, many potentially sound projects have failed to produce much, either in the form of salmon or understanding. In the circumstances of expanding manpower requirements, and considerable mobility of professionals, it has not been uncommon for a project to be conceived by one person, constructed by a second, evaluated by a third who turned his responsibilities over to a fourth, and so on. It is literally true that on many projects there have been more generations of investigators than generations of salmon. In consequence, there have been many opportunities for forgetfulness about the original objectives, and frequent erosion of the awareness of sources of error in whatever data were collected. For the large proportion of salmon-enhancement projects, it is not scientifically rewarding to go back over the record. It should be appreciated that enhancement projects should not be undertaken without a clear plan beforehand for a high-class investigation of their effectiveness, and that there should be a commitment to continuity to ensure this will occur.

It should also be realized that salmon-enhancement programs will be effective only when they are undertaken in a total administrative complex that involves research components. There are many matters of routine investigation in which it is important to have specialists readily available. For example, a problem on artificial spawning grounds may require the services of a specialist in animal behavior or a specialist in stream algae and bottom organisms, an expert on fish diseases, and so on. Hatchery operations absolutely require the backup of specialists in fish diseases and in nutrition. No program of selective breeding should be undertaken without the advice of a specialist in population genetics. All these kinds of expertise that must be on tap in a total salmon-enhancement program should be part of a local research establishment. It is not efficient to bring in experts from other areas because in the majority of instances local knowledge is a vital ingredient of effective advice.

Perhaps much more important is that research will provide the basis for the enhancement techniques of the future. The potentials of selective breeding, the possibilities of developing off-year pink runs by accelerating maturity in pink salmon and/or by sperm storage, the perfection of techniques of lake and stream fertilization, and so on, are all enhancement possibilities that are the proper subject of research study.

Opportunities for salmon enhancement are enormous. There is enough known now, and a sufficient cadre of experts available, to spend $100 million wisely within the next 3 years. The Fisheries and Marine Service has a "shopping list" of good projects that only requires a decision for financing. The same is true of the International Pacific Salmon Fisheries Commission. The Fisheries Research Station at Nanaimo could readily expand its research on salmon enhancement. For example, the fertilization of Great Central Lake seems to have been a striking research success, having produced larger smolts with high marine survival rates. The next logical step is to enlarge the program to a pilot level, with studies on a dozen coastal lakes in which similar favorable results are indicated. Within 5 years this work could open up an annual expenditure on fertilizers of several hundred thousand dollars. It is to be underlined, emphasized, and repeated until it is realized, that salmon enhancement is a good biological bet, and a necessary biological bet. Nature can be improved upon, and now is a good time to get cracking.

How to do it

If there is validity in the argument that institutions have historic momentum, it would seem inevitable that the money for a salmon-enhancement program would come from the coffers of the federal Treasury Board. And, in so doing, it may be presumed that the tradition of pooling revenues, and dispensing from the pool in relation to the need, would be preserved. In these circumstances, similarly, it would be somewhat inevitable that the Resource Development Branch of the Fisheries and Marine Service (or one of the other development branches of the Service) would take the lead in devising programs and executing them and evaluating them.

There are some obvious disadvantages in going along on this traditional pattern. In the first place, to put it mildly, it does not give the industry and the provincial government, and the fishermen, both sport and commercial, and the general public, a very vivid feeling of participation. Secondly, it runs the risk that a salmon-enhancement program, which requires continuity and singleness of purpose, might suffer from time to time because of the pressures of other aspects of the total activity of fisheries management. Thirdly, it concentrates virtually all of the expertise in the already large technical group in the Fisheries and Marine Service. Finally, it embeds an essentially engineering enterprise that emphasizes construction, in the rather formidable bureaucratic federal matrix which is renowned for its awkwardness in doing simple tasks. For all these reasons, there is merit in considering some novel ways of funding and spending in a major effort at salmon enhancement. For example, it would be useful if the industry were to consider imposing a tax of, say, 1 ¢/lb on commercially caught salmon, with the understanding that this revenue would be earmarked specifically for salmon-enhancement programs. This would realize about $1.5 million per year at present rates of catch. Additionally, a fee collected from saltwater anglers (added to a license fee?) would make another substantial contribution to salmon-enhancement funds. Special grants from federal and provincial governments might further add to the fund.

Once a revenue-producing machinery of this kind is considered, it immediately becomes useful to speculate on the role and membership of an advisory board to the local "chapter" of the Resource Development Branch of the Fisheries and Marine Service. The obvious membership would be representatives of all concerned agencies and public groups — provincial government, fishing industry (companies, cooperatives, unions, fishermen groups), anglers, conservationists, and so on.

It is only a short step further to visualize the creation of a special corporation (with a Board of Directors) that would be charged with the responsibility for conceiving and executing a salmon-enhancement program. If this step were taken, it would be logical to expect a greater emphasis on contracting-out procedures, a greater continuity in the salmon-enhancement program and a highly visible public institution in which action would be uppermost. Thinking of this kind has developed on both the east and west coast. It reflects frustration with present institutional structures and a conviction that more of the same won't help much.

Although this sort of proposal might complicate problems of management, it contains the basically sound idea that enhancement programs should not get stalled in the large set of considerations that guide the priorities within and between government departments. Because salmon take as long as they do to reach maturity, it is essential that a salmon-enhancement program should be long-termed and single-minded in its perspectives. Although it might contradict the contemporary catechisms, a salmon development corporation would clearly recognize these and other facts of life. Moreover, the complications for management may be more apparent than real. Assuming that the necessary legal steps could be taken, the corporation could be responsible for undertaking such things as stream clearance work, construction of engineering works and their maintenance, and the Resource Development Branch could look after the operation of the facilities, once installed. The Branch could presumably carry on a modicum of construction work of its own, and the corporation program would be coordinated accordingly. One way or another the idea could be made to work. The salmon development corporation concept should certainly not be prematurely aborted because of technicalities.

At the very least, the present institutional arrangements should be given a vigorous review. At present there seems to be no well-thought-out program of salmon enhancement for the west coast, except as can be pieced together from the total package of fisheries activity for each of several regions. Research work is not organized as part of a total enhancement program. If $100 million were immediately made available for a salmon-enhancement program, it is doubtful if the Fisheries and Marine Service could spend it — not because there isn't a job to do that they know needs doing and have the experts to do it, but because they don't seem to be organized to do it. It would be encouraging if one could be convinced otherwise, and a review might provide that encouragement.

Regardless of what machinery is eventually set up for whatever salmon-enhancement program is undertaken, it is urgently necessary that provincial government involvement be more substantial than it is at present. With their control over resource management practices, the provincial government is in the vital position in the maintenance of freshwater environments. At present, provincial authorities are bystanders in the salmon business. They have consistently demonstrated substantial awareness of problems of salmon conservation and, from time to time, and in a variety of ways, have been kept informed on federal policy; but the challenges of enhancement go considerably beyond the traditional provincial role of awareness and passive participation. It will be increasingly necessary to make the case that certain streams and certain works that are constructed be given special priority in the deliberations of the provincial water licensing authorities. It will be increasingly necessary to ensure that the impact of other resource uses is minimized by the direct representation of salmon interests in the discussions of committees that are concerned with multiple resource use. Provincial participation in salmon enhancement isn't desirable, it is essential.

Finally, it should also be clearly recognized that a successful salmon-enhancement program will require a complete spectrum of activities that are planned and executed with joint awareness and coordination among agencies. The problem is not made any simpler by the apparent perennial confusion within the Fisheries and Marine Service about the responsibilities of its various groups. No one agency has an exclusive mandate to undertake salmon-development work. As a result, the latent potentials for duplication of effort and gaps in total coverage have continued to be adequately realized, in spite of a considerable effort at liaison and more than a fair amount of goodwill. Even within the various agencies there is considerable confusion about who should be responsible for doing what. It has been popular recently to stress the need for a close tie between "research and development." For the salmon-enhancement business, the coupling should not mean that all research will lead to development, nor that all development will stem from research. What it should mean is that the research conducted should be aimed in the directions that are likely to advise or inspire development; and that development should make full use of research in planning and conducting its programs. This will most likely happen when there is joint planning and budgeting for a large-scale enhancement program, and when research is carried out on the site of existing and new facilities. By so doing, research will be stimulated in appropriate directions, and the application of research to development will be more immediate.

[These topics were vigorously discussed at the workshop in Vancouver in January. There was considerable support for the idea of a Salmon Development Commission, the several Commissioners selected from senior officers of the federal and provincial governments and distinguished citizens with long-established interests in the resource. The Commission would have an Advisory Board representative of the various resource user groups. The work of the Commission would be done by a small staff headed by an Executive Director.

[It would be the job of the Commission to plan an enhancement program, set priorities, award contracts, suggest research activities, and to otherwise facilitate salmon-enhancement work. A 10-year program of $200 million was considered feasible.

[Various funding schemes were discussed, including the idea of a joint federal–provincial loan for a 10-year program, to be paid back over a longer period from tax revenues on salmon landings.

[It remains to be seen which of the ideas generated at the workshop will prove to be viable, but there was little doubt that something could be worked out.]

Conclusion

There is always a chance that, in a document this long, a writer may get so involved that his main point doesn't come through. To be sure this hasn't happened, it is here stressed that the main point is: now is a good time to invest large sums of money in salmon enhancement in British Columbia. Such an investment is socially desirable, economically rewarding, and biologically sound.

The investment should not be delayed. British Columbia is rapidly changing. Other resource uses and activities are impinging on salmon production, and this can only be prevented to a degree. Accordingly, it is not sufficient to be concerned only with protection. Available salmon populations and habitat should be enhanced to maintain and increase production, starting now.

To do the required job, it is recommended that some special thought be given to some new administrative structures. A salmon development corporation might be a good way to obtain the singleness of purpose and continuity that is needed.

And now, a final postscript. This has not been a technical review, and for a good reason. As the literature on salmon has accumulated, it has increasingly seemed to be more, rather than less, difficult to justify a course of action. As the qualifiers have accumulated, the message has been lost. It has become increasingly easy to find something to be afraid of. Certainly, in their execution, salmon-enhancement projects should ensure that experience elsewhere has been adequately tapped. But a salmon-enhancement program should rest on the conviction that it will pay off. Only then will the necessary continuity and singleness of purpose produce the desired results.

Appendix I

Economic Factors to be Considered Before Undertaking Salmon-Enhancement Programs

BLAKE A. CAMPBELL

Department of the Environment, Fisheries and Marine Service, Vancouver, B.C.

ANY salmon-enhancement program must have economic viability. A particular project can be justified only when accumulative discounted future benefits exceed present and discounted future operating costs. Under normal circumstances the higher the benefit:cost ratio the more justification there is for undertaking an enhancement project.

Use of Benefit: Cost Ratios

One of the basic problems of using benefit:cost ratios for determining the feasibility of any project, and particularly salmon projects, is the time factor. There is a long period between the time when the initial costs are incurred and the benefits are realized. The prices to be applied to future benefits and discount rates to be used in the calculation can, in most cases, determine the feasibility of a project. For salmon projects, the benefits do not start until at least 3–8 years after the initial major construction costs are incurred. Benefit and cost price relationships can and do change during time periods and such changes can completely reverse ratios. The inflationary spiral that has occurred during the past 5 years could not have been anticipated in any benefit:cost analysis that was carried out on salmon-enhancement projects in 1968. The continued unprecedented demand and upward pricing of all food, including salmon, on the domestic and world markets cannot be accurately assessed. Despite such price and cost differences, however, benefit/cost analyses can be valuable at any particular point in time when comparing one salmon-enhancement program with another where the present and future cost/benefit and discount rates used are the same in all calculations.

Benefit:cost ratios are not the only economic measures that should be used in determining the feasibility of a particular enhancement project. There are other important indicators that have to be examined and assessed. Some of these indicators relate to the Canadian economy; others apply only to provincial interests, whereas others relate to particular regions of the province.

Little Effect on World Salmon Production

Salmon-enhancement programs will result in the production of more food but should not be assessed on this basis because there are other and cheaper ways of increasing protein supplies. Salmon is not a staple food product in Canada, but is regarded by many Canadians as an integral part of their diet, even though per capita consumption is relatively low 2–3 lb/capita.

World salmon production has been relatively stable in recent years at between 384,000 and 441,000 metric tons, and at this stage in time any possible increase in salmon production in Canada (total salmon production now 50–60,000 metric tons) as a result of enhancement programs would have relatively little effect on the supply or demand of salmon on the domestic or world markets.

Increased Exports Benefit Canada's Balance of Payments

Normally, the demands of the Canadian domestic market for salmon are satisfied before any salmon is exported. Consumption of salmon in Canada has shown relatively little change in the past 10 years and there is no reason to anticipate any change in our overall food habits in the foreseeable future. Thus, any additional salmon produced as a result of enhancement programs would be sold on the export market where the present and anticipated future demand is high. Dollars earned from these export sales would be a direct benefit to Canada's foreign balance of payments. The countries to which salmon is being exported is also important in terms of the future Canadian economy.

In 1968, salmon and salmon products exported to the United States totalled 9.7 million dollars. Between 1968 and 1971 exports declined but in 1972 rose sharply to 14.3 million dollars as a result of high exports of fall chums and higher prices.

Exports to the United Kingdom also declined from 22.8 million dollars in 1968 to 14.0 million in 1971, but rose sharply in 1971 to 21.3 million dollars. Exports to Japan rose from 8.7 million dollars in 1968 to 15.2 million in 1971 and dropped to 11.0 million dollars in 1972.

Exports to France, Germany, and Sweden also have shown significant increase in recent years. All species and types of salmon and salmon products are in demand on world markets but the Japanese market is particularly interested in importing frozen salmon and salmon roe.

For every additional one million pounds of salmon produced as a result of enhancement projects, the value of exports would be increased between $550,000 and $1.1 million depending on the species and based on 1972 values and utilization. The export value by species is estimated as follows for each one million pounds of raw salmon produced:

Pinks — $550,000; Chum — $600,000; Coho — $75,000; Chinook — $925,000; and Sockeye — $1,000,000.

If 1973 market prices were used in this calculation, the values shown would be at least 25% higher.

Expenditures on Sport Fishing by Americans

In addition to the contributions that increased sales of commercial salmon make to Canadian trade balance, the increased production of certain species of salmon — chinook, coho, and pinks — when produced in areas that result in them becoming available to sports fishermen, would result in the expenditure of additional tourist dollars from the United States. No precise measure is possible of the effect that additional salmon have on actual sport fishing effort but estimates of expenditures by United States fishermen per pound of salmon caught ranges from 7 to $15.

Contribution to Employment

The amount of direct labor required in the production and processing of salmon can be fairly accurately measured. There will be some differences in the amount of labor input dependent on the degree of processing, but these differences are not significant. Between 30,000 and 35,000 man-hours are required in the collecting, transporting, and processing of every one million pounds of salmon. This is equivalent to the employment of an additional 80–100 persons for a 10-week period each year.

In the primary industry and within reasonable limits as a result of the licence-control program that has been in effect in the British Columbia salmon fishery since 1968, no additional men or boats would be required to harvest the additional production that might result from an enhancement project. Higher salmon production, however, means that men and vessels would be working longer periods, operators and crew members would be making higher incomes without too many additional costs.

The increased value of production of every one million pounds of salmon from an enhancement project to the fisherman (based on 1973 prices and including fringe benefits and bonuses) would range from $325,000 for pinks to over $850,000 for chinook. It is estimated that 25–30% of this landed value would be returned to either the federal or provincial governments in the form of direct income taxes from fishermen.

The impact of additional salmon on employment in the secondary industry is more difficult to assess but has to be taken into account in any economic assessment. Material and supplies valued at between $90,000 and $100,000 are required by the fish processing and packing industry for every one million pounds of salmon processed. This includes containers and packaging material, office supplies, and other materials.

Provincial Benefits

The foregoing benefits that are important to the Canadian economy also directly affect the provincial economy. Higher production means higher employment in British Columbia, higher income and the multiplier effect of new wealth created will be more directly felt in British Columbia than in the rest of Canada.

The impact of expenditures by Canadians living in other provinces but visiting British Columbia to take part in more salmon sport fishing will be of direct benefit to British Columbia only for those enhancement programs that result in more salmon being available for recreational use.

Regional Benefits

In assessing the priority of particular enhancement programs consideration should be given to economic needs of different regions in the province.

To stimulate the economy of certain regions of the province, it might be desirable to give priority to enhancement projects that provide employment for persons who have no alternate source of income. Special attention should be given to those projects which will assist Indian fishermen and plant workers to have more opportunity to participate in the salmon fishery. The government is spending one to two million dollars annually through the Indian Fishermen's Assistance Plan on loans and grants to upgrade the Indian fishermen, but unless additional salmon is available to be harvested, this money is being wasted. There are many remote communities that are dependent for their existence on the salmon fishery and the economy of such communities must also be considered in selecting enhancement projects.

Although fishermen are mobile today, local-based fishermen tend to profit most from runs to their home areas. Any enhancement project that provides for the infusion of additional returns to fishermen in such areas as the Queen Charlotte Islands where there are fewer other employment opportunities should have higher priority than a project in more settled areas.

Economics of Different Species of Salmon

The future economic importance of each species of salmon has to be considered when considering enhancement projects.

Sockeye

Up until 1972, nearly all sockeye was used for canning. In 1973 there has been an increasing demand from Japan for frozen sockeye and the price that the Japanese are prepared to pay will influence the amount of sockeye salmon that is diverted from canning use to the fresh and frozen market. The United Kingdom is the traditional export market for canned sockeye but as a result of the entry of the United Kingdom into the European Common Market, Canada will gradually lose its preferential commonwealth tariff (2½%); however, it is doubtful if this action will have any significant effect on sales in the United Kingdom. The opportunity to market frozen sockeye in Japan (in the short term at least) would offset any decline in exports to the United Kingdom. Most sockeye are caught by net fishermen and delivered in the round state and the salmon eggs become available as a valuable by-product.

Pinks

Pink salmon, the smallest salmon because it has a 2-year life cycle, provides the opportunity for earlier benefits. Most pink salmon is canned, although an increased quantity is finding its way to the fresh and frozen market. The size of the pink salmon returning to be harvested has a direct effect on wholesale values. When pinks are very small, the number of pounds of pinks required per case of salmon increases sharply as a higher percentage of the fish is not suitable for canning. In years of extremely large runs, it is often difficult to obtain maximum utilization because of harvesting and transportation problems. As a result a particular run may be underharvested or caught when the quality of the salmon has started to deteriorate. Enhancement programs involving pink salmon must take into consideration the time and place of harvesting.

Coho

Coho salmon are in demand for the domestic and overseas fresh and frozen market. Trollers begin to catch small coho in June while they are still in their growing stage. Many of these early-caught coho are too small and too soft for the frozen market and end up as an inferior canned product. Coho caught by trollers later in the season, closer to maturity, normally have a ready overseas market. France and other European countries have greatly increased coho imports from Canada in recent years. Net-caught coho is normally past the feeding stage and if caught before the effect of fresh water has darkened the skin can be dressed and sold as frozen salmon. Net-caught coho, however, are usually canned. The eggs from net-caught coho are processed into roe and sold to Japan.

Coho from certain salmon-enhancement projects in British Columbia will be a major factor in sport fishing, particularly if the returning coho inhabit the Gulf of Georgia.

Chum

All chum salmon are caught by net fishermen and while this species is white- or pinky-fleshed, there is a good demand on the fresh and frozen market for the early runs of chums. As chums mature and near their spawning rivers, however, the salmon eggs become valuable and ultimately exceed the value of the salmon meat. In 1972, the average market value of salmon roe was $2.27/lb with the average size 10-lb female salmon producing 1½ lb of salmon eggs. The main export market for canned chums is the United Kingdom and Caribbean countries, whereas the eggs are processed and shipped to Japan.

Chinook

Chinook salmon, including both the red- and white-fleshed fish, show a wide range of values depending on the time of harvest. Chinook salmon normally take 4–5 years to mature but are caught by trollers any time after the second year of their life cycle.

Relative prices to troll fishermen (July 1973) at Vancouver show small red chinook (spring) salmon at 85 ¢–$1.05/lb; medium at 1.05–$1.37/lb; and large from 1.45 to $1.65/lb. White chinook salmon are priced much lower: 51–56 ¢ for small; 56–70 ¢ for medium; and 80 ¢–$1.10 for large. Large chinook salmon (over 13 lb) are used for curing and smoking and are very much in demand on the U.S. market.

Chinook salmon that are not taken by troll are usually caught by gillnet en route to their spawning grounds in the major rivers of the province. Prices for net-caught chinook salmon are normally only about half that paid for troll-caught salmon, but in 1973 prices for net-caught red springs are more in line with troll prices. There is a very low demand for net-caught white chinook salmon.

Any enhancement program designed to increase production of chinook salmon should take into consideration the time of harvest of the returning adults. Consideration must also be given if possible to ensuring that red chinook rather than white chinook are produced.

Other Economic Factors

Economic factors relating to the conflicting multiple resource use of water in particular situations have to be mentioned but these are too complex to attempt to resolve at this stage. Any decision on particular enhancement projects must consider these other factors but without very extensive studies it will not be possible to provide adequate assessment.

Values can be placed on projects from the point of view of educational benefits. An enhancement program such as the salmon hatchery on the Capilano River in the Greater Vancouver area must be rated higher because of the educational benefits than the Babine Spawning Channel that is located far from any centers of population or normal tourist routes.

Studies conducted by the Fisheries Service on the Qualicum River Spawning Channel and Control Flow Project on Vancouver Island place the value of this particular project at $1.00 per visitor day. Any costs associated with looking after visitors must be deducted if net benefits are to be assessed.

Appendix II[1]

Salmon Enhancement in Countries Other than Canada

C. H. Clay

Food and Agricultural Organization of the United Nations, Rome, Italy

General Principles

A prerequisite to planning any program of resource enhancement in fisheries is as complete a knowledge as possible of the life history of the fish concerned and the environmental conditions that affect its growth and survival. With this knowledge, an experimental program can be devised to test the effects of changes in the environment and evaluate those that show positive results in terms of larger-scale application. Either with or without such smaller-scale experiments, large-scale applications can be planned to enhance the resource, but experience argues for inclusion of such a preliminary step, as it can lead to eventual economies and improved results. Of equal importance at this stage, because large expenditures may be involved, is a careful analysis of the economics of the program to be undertaken. Such analysis should take into account all of the economic factors, in addition to the social factors as far as it is possible to do so.

A review of the available information from countries other than Canada reveals, as one would expect, that most effort has been put into only the initial step of studying life histories and environment. Somewhat less effort has been put into the second step, and only in two cases known to me has any attempt been made to fulfil the third step of an economic analysis, and even in those cases the analysis has not been as complete as it is possible to make it with knowledge and techniques currently available. Substantial programs of resource enhancement have been undertaken in some countries either without any such assessment, or based only on the best intuitive judgment taking into consideration what little was known or could be guessed of the economic and social consequences, and political considerations.

Hatchery Experience in Europe

Hatcheries in Europe, as in eastern Canada and the United States, have been constructed chiefly for enhancing the resources of Atlantic salmon. In the United Kingdom, as in some other countries, experiments are being conducted in rearing other species artificially. In England, sole, plaice, and turbot have been reared artificially in pilot plant scale operations, and the economics of rearing them to maturity have been examined in a very preliminary way (Palfreman 1973). In Hawaii, mullet have been raised artificially, and in Japan some successful experiments have been conducted on tuna. However, no consideration has been given in any of these cases to the possibility of releasing the fish for later harvest in the wild,[2] so the Atlantic salmon hatcheries would appear at present to be the only form of resource enhancement falling within the scope of this seminar.

It is estimated that there are between 30 and 40 hatcheries for Atlantic salmon in Europe. Of this total, there are definitely known to be 17 in Sweden and 12 in the USSR. The total number of smolts produced is not known, but it is known that Sweden produced about 1.7 million annually in 1970, Finland about 100,000 annually, and Ireland "several hundred thousand" annually. The USSR raises 10 million fry annually but the number raised to smolt stage is not known. If it is about half of the number raised in Sweden, which would seem reasonable, then total European smolt production would be about 3.0 million annually.

At current levels of construction costs it is estimated the total investment in European salmon hatcheries is more than $5,000,000. The cost of raising each smolt is now estimated to be 70¢ or more, depending on interest rates on the investment, etc. Improvement in diet and mechanization has steadily increased efficiency and lowered costs of operation. Costs of operation and construction costs have been well studied and documented. The least well-defined, and in some cases unknown, factor remaining is the percentage return of adults expected from releasing smolts in any given situation. Studies are, of course, continuing, with the aim of maximizing returns by ensuring the smolt size at release is the one that guarantees optimum returns, and that other factors such as timing are also the best. However, there is still a wide range of returns from both hatchery releases and naturally produced smolts, varying from less than 1–10%, and this remains therefore a critical factor in considering hatchery construction as an investment in resource enhancement.

---

[1] This appendix has been prepared from the paper originally written by C. H. Clay. Portions concerning resource enhancement in tropical and subtropical countries have been deleted.

[2] With the exception of the tuna, where the experimenters note the possibility of release of small tuna in the sea as a means of increasing the resource, and apparently use this possibility as a partial justification for continuing their work.

In Europe, as on the east coast of North America, hatchery construction in recent years has been justified on the basis of replacement of natural propagation which has been limited or eliminated by construction of dams, pollution, etc., rather than on the basis of an investment in resource enhancement. Nevertheless, data on costs and returns are becoming more plentiful, and improvements in operation are continuing to the point where consideration might soon be given to construction of hatcheries for Atlantic salmon as an investment project.

Hatchery Experience on the U.S. Pacific Coast

A large number of hatcheries for Pacific salmon have been in existence on the U.S. Pacific Coast for many years. As in Europe, many of these were constructed to replace spawning grounds and rearing areas lost as a result of industrial development, with no real attempt made to justify them economically. Only within the last decade have any detailed studies been made to determine the economic viability of the existing hatcheries. As with the Atlantic salmon hatcheries in Europe, efficiency of operation has been increased steadily, and operating costs have been well known and documented over the years. Within the last decade serious attempts have been made to determine as exactly as possible the return from release of young fish, which is the critical factor economically. Incidental to this, it has been possible to determine at least in gross terms who the beneficiaries of the investment are.

By necessity, the following description and later analysis of what has been done are limited to consideration of the one published record available to the writer at the time of writing. This is the study of returns of fall chinook salmon raised in Columbia River hatcheries, as described by Worlund et al. 1969.

This first detailed study of hatchery returns contains much interesting data, including an analysis which indicates that the benefit:cost ratio for the 1961 brood released is 2.3:1. It also gives an indication as to which major segments of the industry and the sport fishery received the benefits. Some unknown factors are pointed out in the study such as the need to include similar data on coho salmon raised in many of the same hatcheries simultaneously, in order to get a complete picture of the economic viability of the hatcheries studied. In addition, some assumptions are made in determining costs that may be open to question, the chief of these being that no cost is involved to the fishermen in harvesting the extra fish resulting from hatchery releases.

These and other factors are examined in more detail in the following section of this paper, as they relate to investment in resource enhancement.

Atlantic Salmon Hatcheries

Most of the hatcheries for Atlantic salmon built to date have been constructed to help to offset a loss of production caused by various events, and not as investment projects.

Recently, however, an analysis has been made of a proposal for a new hatchery in Iceland, as an investment project. This forms a useful starting point for us to examine the methodology for economic analysis of hatcheries and to define the problems that remain to be solved when dealing with Atlantic salmon.

Costs of hatchery construction and operation are fairly well known, so that in the Iceland proposal it was relatively straightforward to calculate the cost of construction for a hatchery to produce 400,000 smolts annually. This worked out to $736,000 (1970 costs) with construction taking 1 year. The operating costs were estimated to be $111,000 annually, starting the year after construction was completed. Although the hatchery would produce smolts from the first year's operation, no adult return could be expected until the second year of operation, or 3 years after start of construction.

Placing a value on the returns of adult fish involved a number of assumptions. First was the percentage of the smolts that might be expected to survive to return as adults. Based on the wide range of percentages recorded (from less than 1 to over 8%[3]) an average of 5.6% was selected as being possibly attainable under Icelandic conditions. It was decided that one third of these should be allowed to escape to spawn and two thirds would be available for a fishery. It was considered unlikely that any of these would be caught in a foreign fishery such as that in Greenland, so all would be available to be captured in Iceland. Calculating the value of these using average weights and prices, it was found it would be definitely uneconomical to trap them for sale as fresh fish under the assumptions noted. However, the prime interest in increasing salmon production in Iceland is to increase the tourist sport fish industry, and by utilizing the increased numbers of salmon to sell more rod permits, the economics looked more promising. If sufficient numbers of additional salmon could be made available to the sport fishery, the revenue from rod permits could average 1300 Icelandic Kroner or $14.50 per additional adult fish at 1970 exchange rates. This was based on an average of revenue from rod permits presently in existence. On this basis the revenue to be expected from the hatchery operation would be $221,000 annually, commencing the third year after construction started.

I analyzed these data using the method of determining the "Internal Rate of Return," which is felt to be one of the most appropriate for this type of project, and the results are as follows:

---

[3] There were two sets of records considered, one for all ICNAF countries as published in 1970, and one for Iceland alone up to 1969 (unpublished data). Both had about the same range of values.

Year	A[a]	B	C	Balance (C–B–A)	Discount at 10%		12%	
					rate	amt.	rate	amt.
1	736	4	–	–740	.909	–672	.893	–660
2		111	–	–111	.826	– 92	.797	– 88
3		111	221	+110	.751	+ 83	.712	+ 78
4		111	221	+110	.683	+ 75	.636	+ 70
5–25		111	221	+110	5.907	+650	4.806	+530

[a] A = investment cost, B = operating costs, C = returns (all figures in $1000s + benefits–costs.

From this it will be seen that at a discount rate of 10%, benefits exceed cost by $44,000 and at 12%, costs exceed benefits by $70,000, so the internal rate of return is approx. 11%. This is not a particularly attractive rate of return even if interest rates were much lower than they are at present. It must also be recalled that the assumption on rate of return of adults was fairly optimistic, and a sensitivity analysis of this factor would probably reveal that it is the most critical of all factors affecting viability of the project.

U.S. Pacific Coast Chinook Salmon Hatcheries

In examining the benefit/cost analysis for the 1961 brood chinook salmon as described above, it is apparent that the analysis, although considered acceptable for the purposes of the biological paper of which it forms a part, falls short of being a complete benefit/cost analysis in a number of respects.

The most obvious shortcoming is that although costs have been discounted appropriately, the benefits have been added up using the full value at the years in which they occur (Table A II, 1) rather than being discounted to the same time as the costs, as is customary and is one of the basic principles of benefit/cost analysis. A recalculation on this basis indicates that the benefit: cost ratio is reduced from 2.3:1 to 2.0:1, at a discount rate of 5%. At a discount rate of 10%, it is reduced further to 1.78:1, and if the calculation is carried to the point where benefits and costs are equal, the indicated internal rate of return is about 35%. In making these calculations, which are shown in Table A II, 2, it was assumed that the value of the sport fish catch had the same proportionate distribution over the years as the ocean and river commercial catches.

These benefit:cost ratios, and the internal rate of return, although less than the original paper suggests, are still attractive from an investment point of view. But it is considered that some further refinements in the analysis are possible, and should be made to give the best guidance relative to investment in this form of resource enhancement.

These refinements relate to the consideration of timing of construction and operation of the hatchery, and the time required to reach full production and a full return on the investment. The calculations above assumed that both costs and the first year's benefits were attributable to year one of the analysis. It would seem to be more realistic to allow a full year while the costs are being incurred before starting to receive benefits, as we demonstrated in the analysis for Atlantic salmon in the previous section. On this basis, as shown in Table A II, 3, the benefit:cost ratio at 5% discount

TABLE A II, 1. Estimated value of the catch of fall chinook salmon of 1961 brood. (Used as benefits in the b/c analysis.)[a]

Type of Fishery	Age	No. of fish	Wt (*kg*)	Value ($)
Sport		43,985	–	390,147
Ocean Commercial	2	43	78	65
	3	115,755	443,689	425,487
	4	37,441	259,653	349,208
	5	4,797	40,712	58,339
Columbia R. Commercial	2	1,942	5,381	4,272
	3	32,349	263,838	209,389
	4	48,275	571,528	453,592
	5	2,739	34,950	26,504
Total		287,326		1,917,003

B:C ratio

$$\frac{1.917}{831} = 2.3:1$$

[a] Abridged from Table 20, Worlund et al. (1969). Tables 1–5 are based on data contained in this paper.

rate drops to 1.92 and at 10% to 1.5, and the internal rate of return is about 24%.

Furthermore, since the foregoing analyses are somewhat unrealistic in considering only 1 year's costs and the benefits deriving therefrom, two additional calculations were tried, based on the assumption that the capital costs for the 1 year are indicative of the investment required to produce the benefits indicated on a continuing basis over a long period of years. Operating costs were assumed to be the same each year as originally calculated. Two analyses are shown in Table A II, 4 and 5. For a hatchery operating thus over a 20-year period (Table A II, 4) the benefit:cost ratio at 10% discount rate is 2:1, and at 20% discount 1:1, indicating an internal rate of return of about 20%. For a hatchery operating over a period of 50 years (Table A II, 5) the internal rate of return drops to 18%. Both of these calculations involve many assumptions and do not take into account the need for replacement of equipment, etc., which would be necessary over a long-term project, so they can be regarded only as indications of what might be found by a more thorough analysis.

Other Factors in the Benefit/Cost Analysis

There are several factors not included in the foregoing analyses to be noted here. Resource enhancement of the kind described is to date a form of public investment which cannot be analyzed solely on a commercial basis. The direct benefits and costs such as described are a useful means of comparing projects when all other factors are equal, and should carry considerable weight in any overall analysis if done thoroughly. However, with public investment of this type, there are a number of both primary and secondary costs and benefits that should be considered to enable the best choice to be made for use of public funds.

TABLE A II, 2. Discounting of benefits at various rates, with no allowance for construction time. Values in $.[a]

Year	Value at indicated year after cost	Discounted at 5%		Discounted at 10%		Discounted at 35%	
1	5,750	.951	5,460	.909	5,226	.741	4,260
2	797,470	.907	723,300	.826	658,710	.549	437,810
3	1,000,830	.865	865,720	.751	751,623	.406	406,330
4	105,430	.824	86,870	.683	72,008	.301	30,000
Total	1,909,480	Net Benefits	1,681,350		1,487,567		878,400
		Costs	831,520		831,520		831,520
	B:C ratios		2:1		1.8:1		1:1

[a] Approximate internal rate of return = 35%.

TABLE A II, 3. Discounting of benefits at various rates with allowance of one year for construction. Values in $.[a]

Year	Value at indicated year after cost	Discounted at 5%		Discounted at 10%		Discounted at 24%	
1	0	.951	–	.909	–	.806	–
2	5,750	.907	5,210	.826	4,740	.650	3,840
3	797,470	.865	689,810	.751	511,570	.524	417,000
4	1,000,830	.824	824,680	.683	684,560	.423	424,000
5	105,430	.786	82,860	.621	65,470	.341	35,800
Total	1,917,003	Net Benefits	1,602,560		1,266,340		880,640
		Costs	831,520		831,520		831,520
	B:C ratios		1.9:1		1.5:1		1:1

[a] Approximate internal rate of return = 24%.

Table A II, 4. Calculation of internal rate of return of theoretical hatchery with continuing costs and benefits and project life of 20 years, with benefits commencing in year 2. (Amounts in $1000.)[a]

Year	Capital Cost(A)	Operating Cost(B)	Returns (C)	Balance (C–A–B)	Discount at 10%		Discount at 20%	
1	3920[b]	–	–	–3920	.909	–3563	.833	–3265
2		557[b]	5	–552	.826	–455	.694	–383
3		557	802	+245	.751	+184	.579	+141
4		557	1802	+1245	.683	+850	.482	+600
5		557	1907	+1350	.621	+838	.402	+542
6–20		557	1907	+1350	4.723	+6376	1.879	+2537

$$\text{B:C} = \frac{+8248}{-4018} = 2{:}1 \qquad \text{B:C} = \frac{+3820}{-3648} = 1{:}1$$

[a] Capital cost arrived at by assuming ratio of capital cost to operating cost for Klickitat hatchery (1:2) is indicative of overall ratio. Applying this ratio to total costs of $831,522, the capital portion is $274,400. This is 7% (for 1 year's operation) of the total capital cost, which is therefore $3,920,000, and operating costs are $831,522 less $274,400 or $557,000 annually.

[b] Internal rate of return = 20% approx.

Table A II, 5. Calculation of internal rate of return of theoretical hatchery with continuing costs and benefits and project life of 50 years, with benefits commencing in year 3. (Amounts in $ × 1000.)[a]

Year	Capital Cost(A)	Operating Cost(B)	Returns (C)	Balance (C–A–B)	Discount at 10%		Discount at 18%	
1	3920	–	–	–3920	.909	–3563	.847	–3320
2		557	–	–557	.826	–460	.718	–400
3		557	5	–552	.751	–415	.609	–336
4		557	802	+245	.683	+167	.516	+127
5		557	1802	+1245	.621	+773	.437	+544
6–50		557	1907	+1350	6.124	+8267	2.427	+3280

$$\text{B:C} = \frac{+9{,}207}{-4{,}438} = 2{:}1 \qquad \text{B:C} = \frac{+3{,}951}{-4{,}056} = 1{:}1$$

[a] Internal rate of return approx. 18%.

Among the primary costs are: 1, the cost of research and investigations needed to bring a hatchery to the state where it can produce returns of the magnitudes described; 2, the real cost of harvesting the fish resulting from the hatchery.

Among the primary benefits are: 1, increased utilization of fishing vessels and other harvesting and processing equipment where such facilities are presently underutilized; 2, increased hours or days of employment in the fishing industry, where fishermen and other workers are presently underemployed.

Secondary benefits to be considered might include those arising from increased sales of sport fishery gear and equipment, increased tourist revenues, and increased sales of goods and services to the fishing industry.

Finally, in addition to considering all the possible benefits and costs and evaluating as many as possible in monetary terms, some consideration must be given to the risk involved in any new investment proposal. In the case of salmon hatcheries the most important risk is that some natural phenomenon may affect the survival of the fish during the period they are in the wild state. As pointed out earlier, the rate of return of adults is a critical factor to the economics of the investment. It is particularly important that returns are high as early as possible after the investment, and the risk involved in low early returns should be considered.

The Problem of How the Benefits Should be Distributed

With the risks inherent in hatchery construction for resource enhancement, it is difficult to see how financing could be arranged from other than public funds, except perhaps in certain very special circumstances. But a further and more important reason for not financing privately is the difficulty of ensuring that the investor receives the maximum (or any) return on his investment. For Pacific coast salmon, there would seem to be no way of developing increased adult returns without them being subjected to the various types of sport and commercial fisheries through which they pass in their migrations. It seems apparent that each location will differ in the problems surrounding this aspect, so that if it is desired to recover for the public treasury some part of the investment costs, exact studies will have to be made of where the benefits accrue to consider whether or how to set up a system of fees or licences or other means to provide revenue commensurate with these benefits.

Conclusions

Although the analyses included in the foregoing sections are incomplete in many respects, it is apparent that salmon hatcheries offer potentially good investment possibilities in Canada based on experience in other countries. It is also indicated that thorough analysis with presently available techniques can provide a basis for deciding which projects are viable and will yield sufficiently attractive returns to the country to make investment worthwhile. It is recommended that in developing a program of resource enhancement all projects be analyzed in as much detail as possible and utilizing all of the latest techniques available for benefit:cost appraisal.

It is also recommended that further research and investigations be channelled so as to provide the information needed to make possible the type of detailed analyses described. Priority should be given to research and studies that would permit prediction of adult returns with greater accuracy. These should include studies of factors that influence the rate of return, such as size at time of release, timing of release, etc. Studies of the benefits applicable to each type of fishery, both primary and secondary, should be encouraged, as well as more detailed studies of costs.

Although investment decisions on resource enhancement can and are being made now, they could be greatly improved by such further intensified studies, which require collaboration among biologists, engineers, and economists.

FAO. 1973. Fishery Harbour Planning. Fisheries Technical Paper No. 123. FIIV/T123.

Netboy, A. 1968. The Atlantic Salmon, a Vanishing Species? Faber and Faber, London.

Palfreman, D. A. 1973. The Economics of Marine Fish Farming. Fish Farming Int. 1: 47–52.

UNDP. 1970. Iceland — Salmon Hatchery at Lake Myvatn. Report of the Administrator of UNDP. No. ICE/IFS/1.

Worlund, D. D., R. V. Wahle, and P. D. Zimmer. 1969. Contribution of Columbia River Hatcheries to Harvest of Fall Chinook Salmon. Fish. Bull. 67: 361–391.

CORRECTIONS TO RECENT PUBLICATIONS

CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 30, No. 6 — paper by P. J. Colby and L. T. Brooke: *Effects of temperature on embryonic development of lake herring (Coregonus artedii).*

Page 807: Figure 3, label for Y-axis should read "Development rate × 10^3" instead of "Development rate × 10^{-3}."

VOLUME 30, No. 10 — note by E. Scherer and S. Nowak: *Apparatus for recording avoidance movements of fish.*

Page 1594: Right column, insert "potentiometer. This voltage is simultaneously fed (1)" between lines 14 and 15. Correction has been made in the reprint of this note.

VOLUME 30, No. 11 — paper by E. B. Davidoff, R. W. Rybicki, and K. H. Doan: *Changes in the population of lake whitefish (Coregonus clupeaformis) in Lake Winnipeg from 1944 to 1969.*

Page 1678: Right column, line 17, "$Y = 0.7165 + 0.0032X$" should read "$Y = 0.7165 + 0.00032X$." Correction has been made in the reprint of this paper.

VOLUME 31, No. 1 — paper by M. E. Markiw and K. Wolf: *Myxosoma cerebralis: isolation and concentration from fish skeletal elements — sequential enzymatic digestions and purification by differential centrifugation*

Page 16: Right column, lines 22–30 should be placed after line 50.

COMING NEXT MONTH/ À PARAÎTRE LE MOIS PROCHAIN

Determination of the apparent complexing capacity of lake waters **Y. K. Chau, R. Gächter, and K. Lum-Shue-Chan**

Effect of acclimation temperature and heat shock on vulnerability of fry of lake whitefish (*Coregonus clupeaformis*) to predation **T. G. Yocum and T. A. Edsall**

Relative importance of additive genetic and maternal sources of variation in early survival of young splake hybrids (*Salvelinus fontinalis* × *S. namaycush*) **G. B. Ayles**

Influence of a thermal effluent on movement of brown bullhead (*Ictalurus nebulosus*) as determined by ultrasonic tracking **J. R. M. Kelso**

Reduction of Atlantic salmon (*Salmo salar*) catches in Canada attributed to the Greenland fishery **J. E. Paloheimo and P. F. Elson**

Fish sporozoa: extraction of antigens from *Myxosoma cerebralis* spores which mimic tissue antigens of rainbow trout (*Salmo gairdneri*) **G. B. Pauley**

Factors influencing the seasonal and daily patterns of alewife (*Alosa pseudoharengus*) migration in a Rhode Island river **W. A. Richkus**

Long-distance migration of an Atlantic mackerel (*Scomber scombrus*) **L. S. Parsons and J. A. Moores**

Notes

Coliform flora of migrating sockeye salmon (*Oncorhynchus nerka*) **G. A. Strasdine and L. Dubetz**

First record of the American shad (*Alosa sapidissima*) from Labrador waters **G. M. Hare and H. P. Murphy**

Toxicity of phosphamidon to American lobsters (*Homarus americanus*) held at 4 and 12 C **D. W. McLeese**

Is morpholine an effective olfactory stimulant in fish? **T. J. Hara**

Empirical functions relating metabolic processes in aquatic systems to environmental variables **R. A. Parker**

The fish size, prey size, handling time relation in several sunfishes and some implications **E. E. Werner**

Peptone beef extract glycogen agar, a selective and differential aeromonas medium **R. H. McCoy and K. S. Pilcher**

Bilateral hyperplasia of the thymus in a rainbow trout (*Salmo gairdneri*) **J. F. McArdle and R. J. Roberts**

Partial replacement of herring meal with soybean meal and other changes in a diet for rainbow trout (*Salmo gairdneri*) **C. Y. Cho, H. S. Bayley, and S. J. Slinger**

Acute toxicities of a polychlorinated biphenyl (PCB) and DDT alone and in combination to early life stages of coho salmon (*Oncorhynchus kisutch*) **M. T. Halter and H. E. Johnson**

Spectrophotometric determination of dissolved oxygen concentration in water **W. S. Duval, P. J. Brockington, M. S. von Melville, and G. H. Geen**

Relationship of fishing mortality to natural mortality at the level of maximum sustainable yield under the logistic production model **R. C. Francis**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed below.

(1) Department of the Environment
Fisheries and Marine Service
Pacific Biological Station
P.O. Drawer 100
Nanaimo, British Columbia V9R 5K6

(2) Department of the Environment
Fisheries and Marine Service
Vancouver Laboratory
6640 N.W. Marine Drive
Vancouver, British Columbia V6T 1X2

(3) Department of the Environment
Fisheries and Marine Service
Pacific Environment Institute
4160 Marine Drive
West Vancouver, British Columbia V7V 1N6

(4) Department of the Environment
Fisheries and Marine Service
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba R3T 2N6

(5) Department of the Environment
Fisheries and Marine Service
Arctic Biological Station
P.O. Box 400
Ste. Anne de Bellevue, Quebec H9X 3L6

(6) Department of the Environment
Fisheries and Marine Service
Biological Station
St. Andrews, New Brunswick E0G 2X0

(7) Department of the Environment
Fisheries and Marine Service
Marine Ecology Laboratory
Bedford Institute
P.O. Box 1006
Dartmouth, Nova Scotia B2Y 4A2

(8) Department of the Environment
Fisheries and Marine Service
Halifax Laboratory
P.O. Box 429
Halifax, Nova Scotia B3J 2R3

(9) Department of the Environment
Fisheries and Marine Service
Biological Station
St. John's, Newfoundland A1C 5X1

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants.

(1) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Station biologique du Pacifique
C.P. 100
Nanaïmo (Colombie-Britannique) V9R 5K6

(2) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire de Vancouver
6640 nord-ouest, prom. Marine
Vancouver (Colombie-Britannique) V6T 1X2

(3) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Institut de l'environnement du Pacifique
4160, prom. Marine
Vancouver-Ouest (Colombie-Britannique)
V7V 1N6

(4) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Institut des eaux douces
501. University Crescent
Winnipeg (Manitoba) R3T 2N6

(5) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Station biologique de l'Arctique
C.P. 400
Sainte-Anne-de-Bellevue (Québec) H9X 3L6

(6) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Station biologique
St. Andrews (Nouveau-Brunswick) E0G 2X0

(7) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire d'écologie marine
Institut de Bedford
C.P. 1006

Dartmouth (Nouvelle-Ecosse) B2Y 4A2

(8) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Laboratoire d'Halifax
C.P. 429
Halifax (Nouvelle-Ecosse) B3J 2R3

(9) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Station biologique
Saint-Jean (Terre-Neuve) A1C 5X1

(10) Department of the Environment
Fisheries and Marine Service
Office of the Editor
116 Lisgar Street
Ottawa, Ontario K1A 0H3

(11) Department of the Environment
Fisheries and Marine Service
Canada Centre for Inland Waters
Great Lakes Biolimnology Laboratory
P.O. Box 5050
867 Lakeshore Rd.
Burlington, Ontario
L7R 4A6

(12) Department of the Environment
Fisheries and Marine Service
Atlantic Regional Office, Research
1 Sackville Place
P.O. Box 159
Halifax, Nova Scotia B3J 2M4

(10) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Bureau du rédacteur
116, rue Lisgar
Ottawa (Ontario) K1A 0H3

(11) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Centre canadien des eaux intérieures
Laboratoire de biolimnologie des Grands lacs
C.P. 5050
867, ch. Lakeshore
Burlington (Ontario) L7R 4A6

(12) Ministère de l'Environnement
Service des pêches et des sciences de la mer
Bureau régional de l'Atlantique, recherche
1 Place Sackville
C.P. 159
Halifax (Nouvelle-Ecosse) B3J 2M4

Recent Studies on Investigations Published in Other Periodicals

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études sur des recherches mentionnées dans d'autres périodiques

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'étude, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

1944. Zitko, V., and P. M. K. Choi. 1973. Oral toxicity of chlorinated dibenzofurans to juvenile Atlantic salmon. Bull. Environ. Contam. Toxicol. 10: 120–121. (6)

1945. Margolis, L., R. L. Rausch, and E. Robertson. 1973. *Diphyllobothrium ursi* from man in British Columbia — first report of this tapeworm in Canada. Can. J. Public Health, Nov.–Dec., p. 588–589. (1)

1946. Healey, F. P. 1973. Characteristics of phosphorus deficiency in *Anabaena*. J. Phycol. 9: 383–394. (4)

Interpretive Articles Series

This new series commenced in 1966. The articles interpret work already published in primary journals. They appear at irregular intervals in various technical publications. Frequently an article essentially duplicates, condenses, or is based on an article in another series.

Articles explicatifs

Cette série a débuté en 1966. Les articles donnent une explication de travaux déjà publiés dans des revues prioritaires. Ils paraissent à intervalles irréguliers, dans diverses publications techniques. Souvent, un article se basera sur un écrit d'une autre série, le condensera ou le reproduira presque tel quel.

303. Mann, K. H. 1972. The coastal zone, its basic ecological properties and subdivisions, p. 123–131. *In* Coastal zone, Vol. 1. Selected background papers, seminar at Bedford Institute of Oceanography, Dartmouth, N.S., March 1972. (7)

304. Platt, T. 1972. Problems of modelling coastal zone ecosystems, p. 137–149. *In* Coastal zone, Vol. 1. Selected background papers, seminar at Bedford Institute of Oceanography, Dartmouth, N.S., March 1972. (7)

Manuscript Report Series

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

Rapports manuscrits

La série comprend des articles d'information et des rapports préliminaires non publiés, qui ne sont pas destinés à la distribution générale. Ils ne doivent pas être donnés en références sans l'assentiment du service dépositaire et sans une indication claire de leur forme manuscrite.

STOCKNER, J. G., AND H. D. SMITH. 1974. Chemical studies of the recent sediments of Babine Lake British Columbia. Fish. Res. Board Can. MS Rep. 1268: 20 p. (3)

FORRESTER, C. R., D. E. CHILTON, AND J. E. SMITH. 1973. Length and age composition of English sole (*Parophrys vetulus*) in commercial landings from North Hecate Strait, British Columbia 1953–1971. Fish. Res. Board Can. MS Rep. 1269: 43 p. (1)

PARKER, R. R., J. SIBERT, B. A. KASK, T. BROWN, B. SMYTHIES, AND D. CUMMING. 1973. Chemical observations on the waters of Alberni Inlet, 20 March, 1973. Fish. Res. Board Can. MS Rep. 1270: 95 p. (1)

Technical Report Series

Technical Reports are research documents that are of sufficient importance to be preserved, but which for some reason are not appropriate for primary scientific publication. Inquiries concerning any particular Report should be directed to the issuing establishment.

Rapports techniques

Les rapports techniques sont des documents de recherche qui revêtent une assez grande importance pour être conservés mais qui, pour une raison ou pour une autre, ne conviennent pas à une publication scientifique prioritaire. Pour toute demande de renseignements concernant un rapport particulier, il faut s'adresser au service responsable.

424. HARLING, W. R., D. DAVENPORT, M. S. SMITH, A. C. PHILLIPS, AND S. J. WESTRHEIM. 1973. *G. B. Reed* groundfish cruise no. 73–2, September 5–25, 1973. Fish. Res. Board Can. Tech. Rep. 424: 37 p. (1)

425. TOMLINSON, N., S. E. GEIGER, G. A. GIBBARD, AND S. J. WESTRHEIM. 1973. Utilization of Pacific rockfish. 1. Comparison of *Sebastes alutus*, *S. reedi*, and *S. proriger* with respect to their quality during chilled and frozen storage. Fish. Res. Board Can. Tech. Rep. 425: 31 p. (2) (1)

Translation Series

The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. A limited number of copies are available for distribution as long as the supply lasts, or may be obtained by interlibrary loan, from the station where the translation has been deposited. Information on translations generally may be obtained from the Office of the Editor.

Traductions

Les traductions dont il est question ci-après ont été préparées pour ou par le Service des pêches et des sciences de la mer, à des fins de recherche. On peut en obtenir un nombre limité d'exemplaires ou se les procurer par prêts entre bibliothèques, aux stations dépositaires de ces textes. On peut en général se renseigner sur ces traductions au Bureau du rédacteur.

Margalef, R. 1957. New aspects of the problem of the suspension of planktonic organisms. Invest. Pesq. 7: 105–116. (Transl. from Spanish by Fish. Res. Board Can. Transl. Ser. No. 2914, 1974) (1)

Gritsevskaya, G. L. 1967. River hydrography of the west coastland of the White Sea. Tr. Karel. Otd. GosNIORKh 5: 10–17. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2915, 1974) (4)

Tiller, I. V. 1972. Age and growth rate of three-spine stickleback (*Gasterosteus aculeatus* L.) in Lake Dal'nee. Izv. Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (TINRO) 82: 219–225. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2916, 1974) (1)

Kalninya, Z. K. 1967. Zooplankton in 55 lakes of the northern Latvian SSR. Tr. Karel. Otd. GosNIORKh 5: 134–139. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2917, 1974) (4)

Maistruk, P. N., L. N. Kononko, G. I. Solomko, L. V. Shapovalova, and G. N. Nikolaeva. 1973. The effect of krill paste on some indices of protein metabolism. Vrach. Delo 4: 5–7. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2918, 1974) (8)

Guseva, K. A. 1968. On the role of water agitation in the periodicity of development of plankton diatoms. Gidrobiol. Zh. 4(3): 3–8. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2919, 1974) (4)

Turdakov, A. F. 1970. Behaviour of trout spermatozoa in Ringer solutions of varying concentrations. Nauchn. Dokl. Vyssh. Shk. Biol. Nauki 13(2): 20–24. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2920, 1974) (1)

Nikolaeva, E. T. 1972. The size–weight characteristic and feeding of chum salmon fry in the mouths of the Kamchatka Rivers. Izv. Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (TINRO) 82: 153–164. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2921, 1974) (1)

Yamanaka, H., M. Takamizawa, and K. Amano. 1973. Relation between the colour of tuna meat and the activity of metmyoglobin reductase — I. A determination method of metmyoglobin reductase activity of the extract from tuna meat. Nihon Suisan Gakkai-Shi 39: 667–671. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2922, 1974) (2)

Yamanaka, H., M. Takamizawa, and K. Amano. 1973. Relation between the colour of tuna meat and the activity of metmyoglobin reductase — II. Changes in the activity of metmyoglobin reductase during frozen storage and subsequent thawing of tuna meat. Nihon Suisan Gakkai-Shi 39(6): 673–681. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2923, 1974) (2)

Tanaka, K. 1973. Experiment on natural tocopherol used for frozen fish as an antioxidant. Reito 48(548): 499–504. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2924, 1974) (8)

Lapin, V. I. 1973. A comparative study of the fat content and qualitative composition of lipids in the white and black sea flounders (*Platichthys flesus* L.). Biol. Nauki 16(3): 41–48. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2925, 1974) (8)

Yarvekyul'g, A. A. 1967. Ostracoda in Karelian lakes. Tr. Karel. Otd. GosNIORKh 5: 205–210. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2926, 1974) (4)

Ipatov, V. V. 1972. The dynamics of the lipoprotein content in the blood serum of Baltic cod. Tr. Vses. Nauchno-Issled. Inst. Morsk. Rybn. Khoz. Okeanogr. (VNIRO) 85(3): 68–73. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2927, 1974) (9)

Karnicka, B., and I. Jurewicz. 1971. Changes in the content of trymethylamine oxide, trimethylamine and total volatile basic nitrogen in fresh and frozen fish. Pr. Morsk. Inst. Rybackiego 16(B): 193–203. (Transl. from Polish by Fish. Res. Board Can. Transl. Ser. No. 2928, 1974) (9)

Nikolaeva, E. T. 1972. Trophic requirements and growth of young Kamchatka chum salmon. Izv. Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (TINRO) 82: 165–178. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2929, 1974) (1)

Guryanova, Ye. F. 1962. Scub shrimps (*Amphipoda gammaridea*) of the northern part of the Pacific Ocean (Part 1). Opred Faune SSSR, Akad. Nauk SSSR 74: 1–440. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2930, 1974) (10)

Mamuro, H., A. Kato, S. Suzuki, and N. Matsutani. 1973. Problems in the gas chromatographic analysis of the fatty acids composition of soybean oil. Yukagaku 22: 442–445. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2931, 1974) (8)

Takagi, T., M. Yamazaki, K. Fukuzuki, T. Akiya, and M. Aoyama. 1973. Gas chromatographic analysis of BHT, BHA and heat transfer media in fatty oils by precolumn method. Yukagaku 22: 357–362. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2932, 1974) (8)

Ovchinnikov, V. V., G. Z. Galaktionov, R. I. Polonnikov, and L. A. Ershov. 1971. On the orientation of the European freshwater eel (*Anguilla anguilla* L.) during the migration period. Atlant. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (AtlantNIRO) 36: 11–23. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2933, 1974) (6)

Platoshina, L. K. 1972. A description of the condition of the hypophyses of fall chum salmon in the Amur River in different sectors of the spawning migration. Izv. Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (TINRO) 77: 233–235. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2934, 1974) (1)

Igarashi, H., K. Zama, and A. Ishigaki. 1953. On the study of a whale (*Ziphius cavirostris*, Cuvier) head blubber oil. Nihon Suisan Gakkai-Shi 18: 493–496. (Transl. from Japanese by Fish. Res. Board Can. Transl. Ser. No. 2935, 1974) (8)

Khodorkovskii, V. A., and R. I. Polonnikov. 1971. On the study of ultraweak magnetic receptions in fish. Atlant. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (AtlantNIRO) 36: 72–77. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2936, 1974) (1)

Kharkevich, N. C. 1967. Properties of organic matter of the waters of southern Karelia. Tr. Karel. Otd. GosNIORKh 5: 59–65. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2937, 1974) (4)

Rosly, Yu. S. 1972. The effect of life conditions on the abundance of young Amur chum salmon in rivers. Izv. Tikhookean. Nauchno-Issled. Inst. Rybn. Khoz. Okeanogr. (TINRO) 77: 134–143. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2938, 1974) (1)

Razvozzhaeva, E. A., B. V. Petrov, and N. A. Tyukavkina. 1973. The carboxylic acid esters in metamorphic rocks of the Patomsk Highland. Geokhimiya 5: 790–796. (Transl. from Russian by Fish. Res. Board Can. Transl. Ser. No. 2939, 1974) (8)

Giere, O. 1970. Investigations of microzonation and ecology of marine Oligochaeta in the shallows of the Isle of Sylt (Germany). Veröff. Inst. Meeresfrosch. Bremerhaven 12: 491–529. (Transl. from German by Fish. Res. Board Can. Transl. Ser. No. 2940, 1974) (1)

The Fourteenth Supplement to List of Articles in the Translation Series No. 2301–2825, with Indexes to Authors and Translators, is now available from the Department of the Environment, Fisheries and Marine Service, Office of the Editor, 116 Lisgar St., Ottawa, Ont. K1A 0H3.

Le quatorzième supplément de la liste des articles dans la série des traductions n° 2301–2825, avec index d'auteurs et de traducteurs, est maintenant disponible au Ministère de l'Environnement, Service des pêches et des sciences de la mer, Bureau du directeur de la publication, 116 rue Lisgar, Ottawa, Ontario K1A 0H3.

ADDITIONAL PUBLICATIONS / PUBLICATIONS ADDITIONNELLES

The Service also publishes a series of *Bulletins*, each on a specific topic. These are for sale by Information Canada, Ottawa, and remittances must be in advance, payable in Canadian funds to the order of the Receiver General of Canada. *Bulletins* are also available from local booksellers and Information Canada bookshops throughout Canada.

The *Annual Report* of the Board and the biennial *Review* of investigations are available free, on request. All publications may be referred to at establishments located at: Ottawa, Ont.; Nanaimo, Vancouver, and West Vancouver, B.C.; Winnipeg, Man.; Ste. Anne de Bellevue, Que.; St. Andrews, N.B.; Halifax and Dartmouth, N.S.; and St. John's, Nfld.

Le Service publie également une série de *Bulletins*, chacun traitant sur un sujet spécifique. Les publications de l'ORP sont vendues par Information Canada à Ottawa. Les remises, payables d'avance en monnaie canadienne, doivent être établies à l'ordre du Receveur général du Canada. Les *Bulletins* sont aussi en vente chez votre libraire et dans les librairies d'Information Canada.

Vous pouvez obtenir le Rapport Annuel et la Revue biennale gratuitement. On peut consulter ces ouvrages aux établissements situés à Ottawa (Ont.) à Nanaïmo, à Vancouver, et à Vancouver Ouest (C.-B.), à Winnipeg (Man.), à Ste-Anne-de-Bellevue (P.Q.), à St-Andrews (N.-B.), à Halifax et à Dartmouth (N.-E.), et à St-Jean (T.-N.).

Journal
Fisheries
Research
Board
Canada

Guide to Authors

A more detailed guide on preparation of manuscripts from theses is available from the Office of the Editor.

EDITORIAL PRACTICES

The *Style Manual for Biological Journals* (3rd ed. American Institute of Biological Sciences, 3900 Wisconsin Ave. NW., Washington, D.C.) is used as the authority in matters of form. The standards for spelling are: *Webster's Third New International Dictionary* and *le Grand Larousse Encyclopédique*. Titles of periodicals are abbreviated as in the *Biological Abstracts* list. References to literature are made by the name-and-year system. The *Journal* follows the names and spelling recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (Third Edition, 1970) Special Publication No. 6 of the American Fisheries Society.

Notes are distinguished from *papers* on the basis of scope or completeness, rather than quality, of the research. They may be on problems with narrow, readily defined limits; on work that is largely confirmatory; on fishing gear or methods, or research techniques; on advances in knowledge arising as by-products of broader studies; on progress in projects that were discontinued; or on preliminary findings that warrant publication before problems are resolved. They may be organized with as much flexibility as papers except that they may have no centered headings. They should take no more than four pages in the *Journal*.

Series titles should be avoided. An *abstract* is required for all manuscripts, and should state concisely in less than 250 words, what was done, the essentials of the new knowledge, and the main conclusions. Abstracts are published in English and French; those submitted in one language only are translated by the *Journal*.

The Editor normally submits each manuscript to at least two *referees*. Contributors are encouraged to suggest at least four names to be added to the list from which referees may be selected.

Illustrations should be submitted in triplicate as photographs (*glossy prints*) no larger than a standard page and must accompany the manuscript. Prints should not be mounted, stapled, or clipped. Symbols, letters and numerals must be neat, and at least 1.5 mm high when the illustration is reduced to the appropriate size. Drawings should, when possible, be grouped to facilitate comparisons.

Proofs are sent to the author, who is responsible for correcting only errors in typesetting. *Alterations to the content must be avoided.*

Fifty *reprints*, without covers, are supplied free. Additional copies may be ordered when the galley proof is returned. A charge of $30.00 per page is made for articles published. When submitting a manuscript, an author should state that his institution will accept this charge.

CHECKLIST ON MANUSCRIPTS

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables or figures and the text, or within the text? Should some of the data be made available separately, in a manuscript report or at a data repository? If you use statistical analysis are the statistics subordinate to the biology?

Would any of the text be clearer if condensed? Are summary statements given at the beginnings of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report?

Does the *abstract* give the essentials of the new knowledge?

Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem?

Are details of *materials and methods* limited to what scientists need in understanding the design of the study and in judging adequacy of the data?

Are generalizations on the *results* adequate? Are findings distinguished from inferences from them?

Is *discussion* limited to interpretation and significance of the findings?

Journal
Fisheries
Research
Board
Canada

Guide des Auteurs

On peut se procurer au bureau du Directeur de la publication un guide plus détaillé pour la préparation de manuscrits à partir de thèses.

DISPOSITIONS GÉNÉRALES

Le *Style Manual for Biological Journals* (3e éd., publié par l'American Institute of Biological Sciences, 3900 avenue Wisconsin N.O., Washington, D.C.) sert de guide quant à la forme de présentation. Le *Webster's Third New International Dictionary* et le *Grand Larousse Encyclopédique* font autorité pour l'orthographe des mots. Les titres de périodiques sont abrégés suivant les *Biological Abstracts*. Les références bibliographiques dans le texte doivent suivre le système nom de l'auteur-année. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (Troisième édition, 1970), Publication spéciale No. 6 de l'American Fisheries Society.

Les *notes* se distinguent des *articles* par la portée et le degré d'achèvement, plutôt que par la qualité des recherches. Les notes peuvent traiter de problèmes à portée restreinte et facilement définissables; de travaux qui en corroborent d'autres; d'engins et de méthodes de pêche, ou de techniques de recherche; de découvertes dérivées d'une recherche de plus grande envergure; de progrès accomplis dans l'exécution de projets abandonnés; enfin, de résultats préliminaires assez importants pour en justifier la publication avant la solution complète du problème. L'agencement des notes peut être aussi flexible que celui des articles, sauf qu'elles ne doivent pas avoir d'en-têtes de paragraphes centrés. La longueur ne doit pas dépasser quatre pages.

Il faut éviter les *articles en série*. Un *résumé* doit accompagner chaque texte et doit exposer brièvement (moins de 250 mots) le travail accompli, les points essentiels des connaissances acquises et les principales conclusions. Les résumés paraissent en anglais et en français, le *Journal* veillant à la traduction dans le cas des présentations unilingues.

Le directeur soumet d'ordinaire chaque texte à l'approbation de deux *arbitres* ou plus. Nous encourageons les collaborateurs à suggérer au moins quatre personnes dont les noms pourraient figurer sur la liste des arbitres.

Les *illustrations* doivent être sous forme de photos (*sur papier glacé*), ne pas dépasser les dimensions d'une page normale et accompagner le texte. Elles ne doivent pas être montées, coupées ni brochées. Les symboles, lettres ou chiffres doivent se détacher avec précision et avoir au moins 1,5 mm de hauteur lorsque l'illustration est réduite à la grandeur voulue. Les dessins doivent, autant que possible, être groupés pour faciliter l'étude comparative.

L'auteur doit revoir les *épreuves* qu'on lui remet et en corriger seulement les erreurs typographiques. *Il doit éviter de changer le contenu du texte.*

Cinquante *tirés a part*, sans couverture, sont fournis gratuitement. L'auteur peut profiter du renvoi des épreuves en placards pour commander des tirés à part supplémentaires. Les frais sont de $30.00 par page pour la publication des articles. Quand un auteur soumet un manuscrit, il doit spécifier que son institution accepte les frais.

LISTE DE CONTRÔLES POUR MANUSCRITS

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les éditeurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes au but de l'étude?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou dans le texte même? Est-ce que certaines données devraient être présentées séparément, dans un rapport manuscrit ou dans un dépôt de données? Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la biologie?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction?

Le *titre* est-il concis, tout en étant représentatif du contenu du rapport?

Le *résumé* donne-t-il l'essentiel des connaissances nouvelles?

L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude? La revue de la littérature se limite-t-elle à la définition du problème?

La section *matériel et méthode* contient-elle le minimum de détails qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données?

Les généralisations sur les *résultats* sont-elles adéquates? Permettent-elles de distinguer entre constatations et déductions?

La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes?

Journal
Fisheries
Research
Board
Canada

Guide for Typists Preparing Copy

Read "Guide to Authors" in the current volume of the *Journal*. When in doubt about how to type or arrange details, follow *Journal* practice.

Type the manuscript on white bond paper, not flimsy, preferably size 21.5 × 28 cm (8.5 × 11 inches). If possible, use paper with the lines numbered in the margin. Leave all margins at least 2.5 cm wide.

Double-space all parts of the manuscript, including tables, captions for illustrations, footnotes, and the reference list. Underline only material that is to be printed in italics. Indent the first line of each paragraph in the text and of each footnote.

In titles and headings, capitalize only the first word and proper names and adjectives. For small capitals, use small letters.

Indicate all text footnotes by superscript arabic numerals. Number them consecutively throughout the manuscript, starting with those on the title page. Place each footnote immediately below the reference to it, separating it from the text by horizontal lines.

On the first page type only the title, the authors' names and affiliations, related footnotes, and the running head (see Appendix A). Type the abstract on a separate page (see Appendix B). Number all pages, beginning with the title page and including those for tables and captions for illustrations.

Have the author check the finished manuscript, and send the ribbon copy and two other clear copies to the Office of the Editor.

Tables: Examine the current volume of the *Journal* to see how tables are set up (see Appendix C).
Note especially the following concerning *numbered tables:*

Type each on a separate page, using paper without the lines numbered.

Number them with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines (see Appendix C).

Illustrations: Send at least 3 *glossy prints* (no larger than 21.5 × 28 cm) of each illustration with the manuscript. Type the author's name and figure number of a strip of paper and fasten it to the back of the print. Do not write or type on the print itself. Also, type the captions in succession on a separate page, headed "Captions for illustrations." Put this page at the end of the manuscript.

Where each figure is first referred to, type in the margin "Fig. — near here."

Mathematical Expressions, Equations, and Formulas: Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulas. In the various expressions, place each component in its correct position (see Appendix D).

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the numeral *one*, and between the capital letter $\overline{\underline{O}}$ and the numeral *zero*. Make other explanatory notes similarly.

References: Check the text to see that all references in the list are referred to. Type the reference list, double-spaced, in the style used in the *Journal* (see Appendix E). Names of journals are abbreviated as in the *Biological Abstracts* list; if an abbreviation is in doubt, type the name in full.

Spellings: About 100 words common in fisheries journals are listed in Appendix F.

Appendices: Appendices A to F are available on request from the Office of the Editor.

Journal
Fisheries
Research
Board
Canada

Guide pour les secrétaires d'auteurs

Lisez le "Guide des Auteurs" reproduit dans l'édition courante du *Journal*. Si vous avez des doutes sur la façon de dactylographier le texte ou d'en agencer les détails, suivez la pratique du *Journal*.

Dactylographiez le manuscrit sur papier blanc de bonne qualité, et non sur papier pelure, mesurant de préférence 21.5 × 28 cm (8.5 × 11 pouces), laissant toujours une marge d'au moins 2.5 cm. Si possible, employez un papier avec lignes numérotées en marge.

Tout le manuscrit, y compris tableaux, légendes de figures, renvois et références bibliographiques, devra être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique. Mettez en retrait la première ligne de chaque paragraphe du texte et de chaque renvoi.

Dans les titres et les en-têtes, n'employez la majuscule que pour le premier mot et les noms propres. Pour les petites majuscules, employez les minuscules du dactylographe.

Indiquez tous les renvois du texte par des chiffres arabes suscrits. Numérotez les renvois consécutivement par tout le texte, à commencer par ceux de la page titre. Placez chaque renvoi directement sous sa référence, le séparant du texte par des lignes horizontales.

La première page ne doit contenir que le titre de la publication, le nom et l'affiliation de ou des auteurs, les renvois appropriés et le titre courant (voir Appendice A). Dactylographiez le résumé sur une page séparée (voir Appendice B). Numérotez chaque page, à commencer par la page titre, et y compris celles qui contiennent les tableaux et la légende des figures.

Voyez à ce que l'auteur vérifie le manuscrit final, et soumettez la première copie et ainsi que deux copies lisibles au bureau du Directeur de la publication.

Tableaux. Consultez une copie du *Journal* pour la composition des tableaux (voir Appendice C).

Portez une attention particulière à ce qui suit au sujet des *tableaux numérotés*.

Dactylographiez chaque tableau sur feuille séparée, vous servant de papier sans lignes numérotées.

Numérotez les tableaux en chiffres arabes.

Tracez des lignes horizontales au-dessus et au-dessous des en-têtes et au-dessous des colonnes, rarement ailleurs; ne tracez jamais de lignes verticales; laissez plutôt des espaces supplémentaires.

Indiquez les renvois dans les tableaux par les minuscules suscrites et placez les renvois au bas du tableau.

Placez les tableaux à la suite des références bibliographiques.

A l'endroit où le tableau est mentionné pour la première fois, indiquez dans la marge "Tableau — près d'ici."

Remarquez que les *tableaux dans le texte* ne sont pas numérotés, sont dactylographiés avec le texte et ne requièrent que rarement des lignes horizontales (voir Appendice C).

Figures: Envoyez avec le manuscrit trois photos *sur papier glacé* (21,5 × 28 cm ou moins) pour chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas à la main ou à la machine sur la photographie même. En plus, dactylographiez les légendes, dans l'ordre, sur feuille séparée, intitulée "Légendes des figures." Intercalez cette feuille à la suite du texte.

A l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge "Fig. — près d'ici."

Equations, formules et expressions mathématiques: Laissez trois espaces au-dessus et au-dessous des lignes renfermant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous d'équations et de formules. Placez chaque composante des différentes expressions dans la position qui lui est propre (voir Appendice D).

Indiquez clairement tous les symboles. Epelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule $\underline{O}$ et le chiffre $\bar{}$*zéro*. Insérez de la même façon toute autre note explicative.

Références: Vérifiez le texte afin de vous assurer que toutes les références apparaissant dans la liste bibliographique sont bien mentionnées dans le texte. Dactylographiez à double interligne dans le style en usage dans le Journal (voir Appendice E). Les noms de revues sont abrégés suivant les *Biological Abstracts*; en cas de doute, écrivez le nom au long.

Orthographe: Une liste d'environ 100 mots communément employés dans les périodiques sur les pêches est donnée en Appendice F.

Appendices: On peut se procurer une copie des Appendices A à F sur demande au bureau du Directeur de la publication.

ANNOUNCEMENT

Availability of Salmon Gonadotropin

A purified gonadotropin preparation (SG-G100) extracted from the pituitary gland of sexually mature chinook salmon (*Oncorhynchus tshawytscha*) is available in quantity from the Vancouver Laboratory Fisheries and Marine Service, at the cost of production of Canadian $3.00 per milligram. Bioassay data are provided with each shipment.

The following references describe the purification procedure and the effective dose in various species:

Donaldson, E. M., F. Yamazaki, H. M. Dye, and W. W. Philleo. 1972. Preparation of gonadotropin from salmon (*Oncorhynchus tshawytscha*) pituitary glands. Gen. Comp. Endocrinol. 18: 469–481.

Donaldson, E. M. 1973. Reproductive endocrinology of fishes. American Society of Zoologists Symposium: The current status of fish endocrine systems. Dec. 29–30 1972. Am. Zool. 13: 909–927.

Requests for this hormone should be addressed to:

Dr Edward M. Donaldson
Vancouver Laboratory
Department of the Environment
Fisheries and Marine Service
6640 N.W. Marine Drive
Vancouver, B.C. V6T 1X2
Canada

Cheques should be made payable to the Receiver General of Canada.

AVIS

Offre de gonadotrophine de saumon

On peut se procurer en quantité une préparation purifiée de gonadotrophine (SG-G100) extraite de l'hypophyse de saumons quinnats (*Oncorhynchus tshawytscha*) matures, au laboratoire de Vancouver du Service des pêches et des sciences de la mer, au coût de production de $3 (monnaie canadienne) le milligramme. Des données de bioanalyse accompagnent chaque envoi.

Suivent des titres d'ouvrages qui décrivent le procédé de purification et la dose efficace pour les diverses espèces:

Donaldson, E. M., F. Yamazaki, H. M. Dye, and W. W. Philleo. 1972. Preparation of gonadotropin from salmon (*Oncorhynchus tshawytscha* pituitary glands. Gen. Comp. Endocrinol. 18: 469–481.

Donaldson, E. M. 1973. Reproductive endocrinology of fishes. American Society of Zoologists Symposium: The current status of fish endocrine systems. Dec. 29–30 1972. Am. Zool. 13: 909–927.

Prière d'adresser les commandes à:

Dr. Edward M. Donaldson
Laboratoire de Vancouver
Ministère de l'Environnement
Service des pêches et des sciences de la mer
6640 N.W. Marine Drive
Vancouver (C.-B.) V6T 1X2

Etablir les chèques à l'ordre du receveur général du Canada.

McTaggart-Cowan, G. I. (Compiler and editor.) 1974. Author–subject index to Fisheries Research Board of Canada Translation Series Nos. 1–1600. Marine Sciences Research Laboratory Technical Reports No. 8: 238 p.

($5.00)

Available from:

Marine Sciences Research Laboratory
Memorial University of Newfoundland
St. John's, Newfoundland

Lists of articles in the Translation Series from 1601–2825, with indexes to authors and translators, are available free from:

Department of the Environment
Fisheries and Marine Service
Office of the Editor
116 Lisgar St.,
Ottawa, Ont. K1A 0H3

Photocopies of the translations are available at .10¢ per page (minimum $1.00) from:

Translation Services
National Science Library
National Research Council of Canada
Ottawa, Ont. K1A 0S2

McTaggart-Cowan, G. I. (compilateur et éditeur) 1974. Index par auteur et sujet des Traductions nos 1 à 1,600 de l'Office des recherches sur les pêcheries du Canada. Rapports techniques du Laboratoire de recherche en sciences de la mer, no 8, 238 p.

($5.00)

Offert au:

Laboratoire de recherche en sciences de la mer
Université Memorial de Terre-Neuve
Saint-Jean (Terre-Neuve)

On peut se procurer gratuitement la liste des articles de la série Traductions, de 1,601 à 2,825, avec index des auteurs et traducteurs, à l'adresse suivante:

Ministère de l'Environnement
Service des pêches et de la mer
Bureau du directeur de la publication
116, rue Lisgar
Ottawa (Ont.) K1A 0H3

On peut se procurer *à* 10¢ la page (minimum de $1.00) des photocopies des traductions *à* l'adresse suivante:

Services de traduction
Bibliothèque scientifique nationale
Conseil national de recherches du Canada
Ottawa (Ont.) K1A 0S2

Bulletins

No. 180. J. L. HART. 1973. Pacific fishes of Canada, 740 p., Fs 94–180, Canada $8.00, Other countries/Autres pays $9.60.

No. 181. THOMAS G. SMITH. 1973. Population dynamics of the ringed seal in the Canadian eastern Arctic, 55 p., Fs 94–181, Canada $2.50, Other countries/Autres pays $3.00.

No. 182. D. H. LORING AND D. J. G. NOTA. 1973. Morphology and sediments of the Gulf of St. Lawrence. 147 p., Fs 94–182, Canada $5.00, Other countries/Autres pays $6.00.

No. 183. W. E. RICKER. 1973. Russian-English Dictionary for students of fisheries and aquatic biology, 428 p., Fs 94–183, Canada $7.00, Other countries/Autres pays $8.40.

No. 184. W. B. SCOTT AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada, 966 p., Fs 94–184, Canada $9.75, Other countries/Autres pays $11.70. (*La version française est en préparation*)

No. 185. KARL BANSE AND KATHARINE D. HOBSON. 1974. Benthic errantiate polychaetes of British Columbia and Washington, 111 p., Fs 94–185, Canada $4.50, Other countries/Autres pays $5.40.

No. 186. P. M. JANGAARD. 1974. The capelin (*Mallotus villosus*): Biology, distribution, exploitation, utilization, and composition, 70 p., Fs 94–186, Canada $3.00, Other countries/Autres pays $3.60.

No. 187. A. GORDON DEWOLF. 1974. The lobster fishery of the Maritime Provinces: Economic effects of regulations, 59 p., Fs 94–187, Canada $3.00, Other countries/Autres pays $3.60.

Available by mail from Information Canada, Ottawa K1A 0S9, and at the following Information Canada bookshops:

Halifax: 1683 Barrington Street
Montreal: 640 St. Catherine Street West
Ottawa: 171 Slater Street
Toronto: 221 Yonge Street
Winnipeg: 393 Portage Avenue
Vancouver: 800 Granville Street

Please make cheques and money orders payable to the Receiver General of Canada. Advance payment is required.

En vente à Information Canada à Ottawa K1A 0S9 et dans les librairies d'Information Canada:

Halifax: 1683, rue Barrington
Montréal: 640 ouest, rue Ste-Catherine
Ottawa: 171, rue Slater
Toronto: 221, rue Yonge
Winnipeg: 393, avenue Portage
Vancouver: 800, rue Granville

Payable à l'avance. Faire les chèques et les mandats payables à l'ordre du Receveur général du Canada.

Journal
of the Fisheries
Research
Board
of Canada

Volume 31, No. 8, August 1974 JFRBAK31(8)1327-1466(1974)

Volume 31, No 8, août 1974

Physiological and ecological significance of the chemical instability of uric acid and related purines in sea water and marine algal culture medium **N. J. Antia and A. F. Landymore** 1327–1335

Descriptions of copepodite stages of *Chiridius gracilis* Farran 1908 (Crustacea: Copepoda) **D. C. Maclellan and C.-T. Shih** 1337–1349

Genetic differences in retention of swimbladder gas between two populations of lake trout (*Salvelinus namaycush*) **P. Ihssen and J. S. Tait** 1351–1354

Taxonomic status of the *Salvelinus alpinus* complex **S. U. Qadri** 1355–1361

Lobster nutrition: the effect on *Homarus americanus* of dietary protein levels **J. D. Castell and S. D. Budson** 1363–1370

Pathophysiology of infectious hematopoietic necrosis virus disease in rainbow trout (*Salmo gairdneri*): early changes in blood and aspects of the immune response after injection of IHN virus **D. F. Amend and L. Smith** 1371–1378

Gravel incubators: a second evaluation on pink salmon, *Oncorhynchus gorbuscha*, including adult returns **R. A. Bams** 1379–1385

Fecundity and gonad observations of the American eel, *Anguilla rostrata*, migrating from Chesapeake Bay, Virginia **C. A. Wenner and J. A. Musick** 1387–1391

Notes

Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*) **A. R. Carlson and R. E. Siefert** 1393–1396

Liver damage in rainbow trout (*Salmo gairdneri*) fed cyclopropenoid fatty acids **Y. Malevski, J. H. Wales, and M. W. Montgomery** 1397–1400

Mixing within experimental enclosures: a cautionary note on the limnocorral **F. M. Boyce** 1400–1405

Phosphoglucomutase polymorphism in brown shrimp, *Penaeus aztecus* **R. R. Proctor, K. T. Marvin, L. M. Lansford, and R. C. Benton** 1405–1407

An isolated population of Arctic char (*Salvelinus alpinus*) inhabiting a warm mineral spring above a waterfall at Cache Creek, Northwest Territories **P. McCart and H. Bain** 1408–1414

Growth and survival of haddock (*Melanogrammus aeglefinus*) larvae in relation to planktonic prey concentration **G. C. Laurence** 1415–1419

Development of steelhead trout (*Salmo gairdneri*) otoliths and their use for age analysis and for separating summer from winter races and wild from hatchery stocks **J. L. McKern, H. F. Horton, and K V. Koski** 1420–1426

Comment on the use of Ivlev's electivity index with planktivorous fish **W. J. O'Brien and G. L. Vinyard** 1427–1429

First record of the margined madtom (*Noturus insignis*) from Canada **P. J. Rubec and B. W. Coad** 1430–1431

Perspectives

Play it again Sam — an essay on salmon enhancement 1433–1456

Corrections to recent publications/Corrections sur récentes publications 1457
Coming next month/A paraître le mois prochain 1459
List of recent publications/Liste de récentes publications 1460–1465
Additional publications/Publications additionnelles 1466
Guide to Authors and Typists/Guide des auteurs et Guide pour les secrétaires d'auteurs *i–iv*

INFORMATION CANADA, OTTAWA, 1974